HANDBOOK OF PLANT BREEDING

Jaime Prohens
Fernando Nuez
Editors

Vegetables II

Fabaceae, Liliaceae, Solanaceae, and Umbelliferae

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Volume 1
Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae
Edited by Jaime Prohens and Fernando Nuez

Volume 2
Vegetables II: Fabaceae, Liliaceae, Solanaceae and Umbelliferae
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Cover illustration: Typical seed production field for an extremely early Japanese onion cultivar (courtesy of M. Shigyo)

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Preface

The production and consumption of vegetables has expanded dramatically in the last years, with a global growth in the production of more than 50% in the last decade, a rate of increase that is much higher than for other plant commodities. Vegetables constitute an important part of a varied and healthy diet and provide significant amounts of vitamins, antioxidants and other substances that prevent diseases and contribute to an improvement in the quality of life. In consequence, it is expected that in the coming years, vegetable crops production will continue its expansion.

Improved varieties have had a main role in the increases in yield and quality of vegetable crops. In this respect, the vegetables seed market is very dynamic and competitive, and predominant varieties are quickly replaced by new varieties. Therefore, updated information on the state of the art of the genetic improvement of specific crops is of interest to vegetable crops breeders, researchers and scholars. During the last years an immense quantity of new knowledge on the genetic diversity of vegetables and the utilization of genetic resources, breeding methods and techniques, and on the development and utilization of modern biotechnologies in vegetables crop breeding has accumulated, and there is a need of a major reference work that synthesizes this information. This is our objective.

The diversity of vegetable crops is appalling, with hundreds of species being (or having been) grown. However, among this plethora of crops, there are some which are prominent, and for which there has been a greater development in the breeding science and development of varieties. In consequence, we have produced two volumes devoted to 20 of these most important vegetable crops. These crops belong to eight different botanical families. Because in many cases crops from the same botanical family share many reproductive, physiological, and agronomic features, as well as similar breeding techniques, we have decide to group them by this taxonomic category. In this respect, this second volume includes 8 chapters that deal with vegetables that belong to four families: Fabaceae or Leguminosae (garden pea, and snap bean), Liliaceae (asparagus, and onion), Solanaceae (eggplant, pepper, and tomato) and Umbelliferae or Apiaceae (carrot).

Chapters have been written by outstanding breeders with wide experience in the crop treated. Each chapter includes information on the origin and domestication, varietal groups, genetic resources, major breeding achievements and current goals of breeding, breeding methods and techniques, integration of the new biotechnologies in the breeding programmes, and the production of seed of specific crops.

The completion of this book would not have been possible without the contributions of the many authors, who have devoted much time to the task of writing the chapters. We also want to thank the staff of Springer, in particular Jinnie Kim and Shoshana Sternlicht, who have made possible to produce a high quality book in a very short time span. We are also indebted to many colleagues for useful suggestions that have contributed to improve this book.

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Garden Pea

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1 Introduction

The variability of the garden pea (*Pisum sativum* L.) and the variety of forms in which it is consumed are a testimony to its long history of cultivation, adaptability and popularity as a crop in countries around the world. The different crop forms are based on different harvest times during the development of either the fruit or the embryo and the presence of particular gene combinations characterize the market product. Those relating to the embryo are those of the fresh vegetable or picked pea, canned, frozen and dehydrated or freeze dried pea markets (Fig. 1 a-d), while those associated with the immature pod are the snow, sugar or mangetout and the sugar snap types (Fig. 1, e and f).

When harvested as young immature embryos while liquid endosperm is still present, peas are rich in vitamins and sugars and appeal to people of all ages. This stage of development is of relatively short duration and is only achieved in large quantities with successional sowings of a single variety or by simultaneous sowing of varieties with staggered flowering times. Both these strategies are utilized by the vining industry, the produce of which is found in both canned and frozen forms where the peas are often graded and of very uniform size. Once embryos have past this stage, they enter the phase of storage product accumulation where starch and proteins are laid down and the levels of sugar decrease. Peas harvested during this phase, when the pod is starting to show signs of starting to dry, are consumed either as fresh vegetable peas or are dehydrated (via either hot-air drying or freeze drying) and used in soups, snacks and other fast foods. Following this stage the accumulation of storage products continues, the embryo starts to loose fresh weight and enters the maturation phase ultimately leading to a dry seed. The dried seed form *per se* is not considered a vegetable but an arable or combinable crop and will not be covered explicitly in this chapter. Being the same species, there are naturally many issues that

are common to both the vegetable and combined crop and references to the latter are mentioned by way of contrasting the two forms and highlighting the differences.

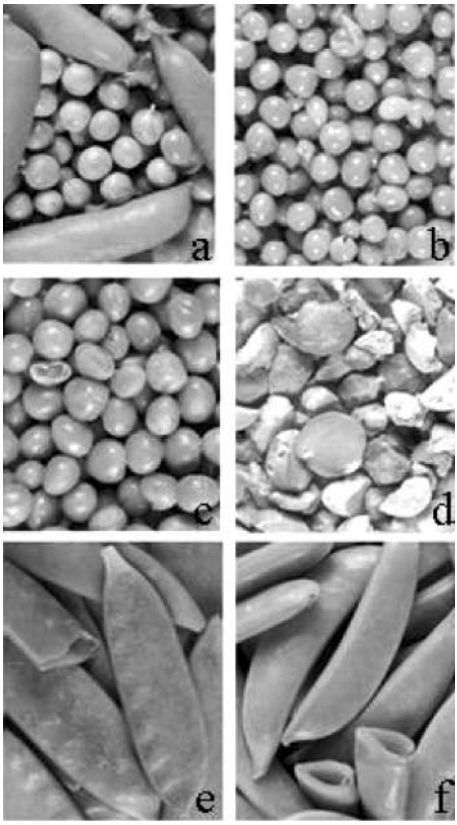

Fig. 1. Forms of vegetable peas. a. fresh picked, b. canned, c. frozen, d. dehydrated, e. mangetout, and f. snap.

References in early herbals (Gerard, 1597) demonstrate that garden peas were a well established by the 1500's and considered something of a premium, particularly the early crops, which are cited as having been transported long distances at great cost to the dining tables of the gentry. There were already a range of distinct plant types at this time 'differing very notably in many respects including 'pease without cods', now referred to as snow peas or mangetout type that were eaten whole and tufted or crowned peas so called because the pods were clustered at the top of the plant rather than in the middle that we now know is as a result of the strong expression of apical fasciation. The popularity of the pea continued and by the end of the 1800's, the vegetable seed list of Sutton's starts with 15 pages devoted to 44 different pea varieties of all classes, illustrating how diverse and popular peas were

within kitchen gardens (Sutton's, 1899). Their persistent popularity even today can be attributed to their relative ease of cultivation and storage, their extended period of harvesting and their taste when freshly picked and cooked. These qualities are appreciated across all cool temperate regions of the word. It is interesting to note that the large-scale production of vegetable peas for the international market which aims to deliver fresh vegetable such as peas to the consumer all year round, is still based largely on old varieties from the early part of the 19th century and is a testament to the variation that has been maintained in cultivation. A wide range of cultivated forms of pea can still be found growing today in gardens and small-holdings in many different parts of the world. Many of these still represent ancient lineage and possible sources of adaptive variation.

2 Origin and Domestication

Pea is an old world cool season annual legume crop who's origins trace back to the primary centre of origin in the near and middle east. Carbonised remains of pea have been found at neolithic farming villages in northern Iraq, southern and south eastern Turkey and Syria and indicate their cultivation and use as food as early as 7000-6000 BC. Their presence is found in remains at sites in Southern Europe soon after (Zohary and Hopf, 1973). While it cannot be proved, it is highly likely that they were consumed in both a fresh vegetable as well as cooked forms. An important secondary centre of diversity for pea is the highland Asiatic region of the Hindukusch that runs the whole length of the southern slopes of the Himalyian mountain range. Distinct forms of cultivated pea from this region include the distinct long vined 'afghan' type and the shorter statured Tibetan ecotype grown on agricultural terraces at high altitudes. Interestingly, examination of germplasm from this region and neighbouring lowland production areas shows clear evidence of the introgression of morphological characters from the high altitude region. The endemic forms of pea from the Transcaucaia and Volga region are a very distinct type with fine foliage and very small seeds (60-80 mg) and are still recognised by some as a separate sub species, *Pisum sativum* spp. *transcaucasicum* (Govorov, 1937). A further secondary centre of diversity includes the central highland region of Ethiopia and uplands of Southern Yemen, which covers the currently known distributional range of *Pisum sativum* ssp. *abyssinicum*. The taxon is well described and distinct from all other *Pisum sativum* forms for a range of morphological characters. Molecular diversity studies have confirmed the narrow genetic variation within known germplasm of this form but also its distinctness from all other sativum forms and postulated it existence as an independent domestication event to that of *Pisum sativum* (Lu et al., 1996; Vershinin et al., 2003). Important novel allelic variation has already been identified within abyssinicum material and this distinct gene pool is currently being explored by groups undertaking wide crosses, mapping and the production of recombinant inbred populations (Weeden et al., 2004).

In these secondary centres are found various forms that demonstrate the wide adaptability of peas to changes in habitat. Numerous expeditions in the 1900's to collect herbarium specimens and germplasm resulted in a wealth of material that

breeders and researchers have been scrutinizing for years. One of the most detailed and authoritative accounts of the genus and classification of forms studies *in situ* is that of Govorov (1937; Gentry, 1974).

The genus *Pisum* comprises of only a small number of taxa. Despite this, the taxonomic literature is far from clear at the level of rank. The most recent review of the *Pisum* taxonomy and ecogeography can be found in Maxted and Ambrose (2001). All taxa within *Pisum* are diploid (2n=14) and the majority are fully intercrossable with a few being more difficult but possible (Ben-Ze'ev and Zohary, 1973). The exact nature of the wild forms that were taken into cultivation and domesticated is impossible to establish unequivocally. Zohary and Hopf, 1973) postulate *Pisum humile* syn. *syriacum* as a possible candidate, as its form closely resembles that of cultivated forms. The evidence emerging from molecular studies has revealed the wide genetic variability within *Pisum elatius* across its distributional range and the presence of material exhibiting characteristics from both *elatius* and *sativum* forms supports the view that there has been frequent introgression between these forms and a better considered as a species complex (Vershinin et al., 2003).

A series of traits that have been associated with domestication are presented in Table 1. A number of these can be considered as prerequisite to the widespread adoption into agrarian practices are those of thin seed coat and non-dehiscent pods. Thin seed coats allows for rapid imbibition and results in more even germination and establishment while non-dehiscent pods, while not essential, as the plants could have been cut prior to full maturity and contained in such a way as to trap the seeds as the pods opened, would have greatly eased the handling and processing of the crop. Rough testa has been associated with domestication (Zohary and Hopf, 1973) but this is not categorical as there are numerous examples of cultivated forms with rough testa eg. Ghatt oasis and the Canary Islands (pers. obs.). The presence of such characters within cultivated material may help in unravelling some of the ancestral forms and lineages within pea germplasm. The seed size of cultivated material has been increased fourfold compared to that of wild material although there is overlap at between the two forms.

Table 1. Domestication traits in wild and cultivated pea and their genetic basis.

Trait	Wild type	Cultivated	Gene basis
Testa surface	Gritty/ rough	Smooth	*Gty*
Testa thickness	Thick, impermeable so slow to imbibe	Thin resulting in rapid imbibition	
Pod Dehiscence	Strongly dehiscent	Non-dehiscent	*Dpo*
Seed size (mg)	60-120	80-550	

Theories into the further spread of peas are gathered from archeological, ethnobotanic and botanical evidence. Along with other crops, their ease of storage, cooking and nutritional properties were key reasons that lead to peas being included in many expeditions and military campaigns and their early dispersal and uptake into other cool temperate regions of the world. The Greeks under Alexander the Great extended their empire eastwards into Mesapotamia and south into Africa and the Romans were responsible for the introduction of cultivated forms into Western

Europe along with many other crop species. The numerous expeditions in the 1900's to primary and secondary centres of origin to collect herbarium specimens and germplasm have resulted in a legacy for breeders and researchers (Gentry, 1974). It is clear however that there are still gaps in our knowledge and coverage and that new locations and populations of wild material are yet to come to light.

3 Varietal Groups

As noted previously, the variation within cultivated peas had been noted and described in numerous references and seed catalogues from the 1700's onwards which was also the time of increasing popularity of the crop. This in itself led to an explosion of new named forms coming to market of which there were clear indications that many were not new forms but just newly renamed. It was not until the early 1900's that systematic cataloguing of cultivated forms was undertaken. Some notable references of this period that detail the characteristics of many hundreds of different varieties and their groupings include the works by Hendrick (1928), Mateo Box (1955), Fourmont (1956) and Sneddon and Squibbs (1958). The primary characters used for grouping varieties relate to seed and pod types, maturity groups and height of the crop and reflected the variation across the market types. The number of groups for which keys were developed varied from anything from 18 to 36 different groups within which were numerous subgroups. Many of these characters are still in general use today but the emphasis is more on specific characters and the descriptor states which, wherever possible are linked to the allelic forms or combinations that underlies that character. A useful point of reference list of characters relating to variation within cultivated forms is the list used in the UPOV guidelines for *Pisum* (Table 2). These form the basis of the distinctness, uniformity and stability test that must be undergone as part of the plant variety rights system (UPOV) and while not covering all the primary characters cited earlier, focuses on those that are highly heritable. The key characters to note from table 2 concerning the various market types of pea are the presence of the *i* allele which results in the peas remaining green rather than their wild type status of yellow. The presence of the *r* and *rb* alleles results in the reduction in starch and higher concentration of sugars found in frozen peas. In the pod types, the presence of the recessive forms of *P* and *V* alleles result in the partial or complete loss of the inner sclerenchyma layer of the pod responsible for giving the pod wall rigidity. Loss of this layer underpins the snow pea or mangetout type. The presence of the recessive allele of the *N* locus results in a thickening of the middle cell layer of the pod resulting in the thicker crunchy textured pods of the sugar snap pea type.

New characters introduced into commercial cultivars since the 1970's represented in the UPOV list are associated with variation in stipule and leaf forms. The first of these is associated with narrow pointed stipules and leaflets that are characteristic of 'rabbit eared' or rouge forms. The genetics of the rogue syndrome which includes a none nuclear component are still not well understood and the character is presently confined to combined dried pea rather than vegetable type. Rogue off types of a range of old vegetable pea varieties have been observed and

isolated but the trait is not seen to offer any advantage to the vegetable pea market so will not be dealt with further. The leaf character that has become widely used in all form of pea breeding since the mid 1970's is the afila gene (*af*) which converts leaflets into tendrils. This character is discussed in more detail in section 5.

Table 2. List of UPOV characters, phenotypic states and associated loci used for grouping varieties.

	Character	Descriptor states	Loci
Seed			
1	Shape of starch grain (cotyledonary chatacter)	Round, wrinkled, dimpled	*R, Rb*
2	Cotyledon colour	Yellow, green, mixed	*I*
		Orange	*Orc*
3	Testa marbling	Brown patterning	*M*
4	Testa anthocyanin	Violet or pink spots, stripes	*F, Fs*
5	Hilum colour	Cream, black	*Pl*
Plant			
6	Anthocyanin colouration	Purple, red to pink	*A, B, Am*
7	Leaf	Leaflets	*Af*
8	Stipule	Small or rudimentary	*St*
9	Stipule	Rounded apex, pointed	*'Rogue syndrome'*
10	Stipule	Flecked, non-flecked	*Fl*
Pod			
11	Pod wall parchment		*P,V*
12	Thickened pod wall		*N*
13	Shape at distal end	Blunt, pointed	*Bt*
14	Colour	Yellow	*Gp*
		Blue-green	*Dp*
		Purple	*Pu, Pur*
15	Intensity of green		*Pa,Vim*

While the UPOV list is useful, it only represents a key for grouping currently registered commercial varieties and so does not cover the wider variation within pea. Neither does it cover useful characters that are based on combinations of genes and an interaction with the environment and thus vary from year to year. The two other primary characteristics referred to earlier that fall into this category are plant height (associated with genes for internode length and an interaction with nodes to flower) and maturity groups (linked to flowering time). Both these characters present problems in quantifying them in practical terms and it is interesting to compare the findings presented in two of the classifications works namely those of Hendrick, (1928) and Sneddon and Squibbs (1958, table 3). Both systems are based on the records obtained for a large number of varieties grown over many years and in the case of Hendrick, many sites. Both reports detail four categories for plant height with Sneddon and Squibbs going so far as to quantify the rage with respect to results obtained in one year and at one location. For maturity groups Sneddon and Squibbs

describe 6 categories whereas Hendrick uses only one (extra early) in his system although in the descriptions of many of the individual varieties, the terms second early and mid season are used.

Table 3. Height and maturity categories in two reference on pea cultivar classification.

	Height categories	Maturity categories
Hendrick (1928)	Very dwarf	Extra early
	Dwarf	
	Medium	
	Tall	
Sneddon and Squibbs (1958)	Dwarf- under 45cm	First early- 63 days*
*results presented for 1953	Dwarf-medium- 45-75cm	Early- 64-67
	Medium- 76-111cm	Second early- 68-71
	Tall- >111cm	Mid season- 72-75
		Late- 76-79
		Very late- 80+

While the UPOV guidelines are important in defining and describing the categories of pea that are cover the variation in commercial material, it is essential for anyone engaging with breeding to know the requirements of the market of their target countries. The registration requirements for peas vary from country to country. A useful survey of requirements across fifteen European countries for agronomic, processing and chemical classes of characters can be found in (Engqvist, 2001) and showed widespread differences for all characters in all classes.

4 Genetic Resources

The inbreeding nature and diploid status of peas and the ease of maintaining fixed inbred lines, together with their wide spread popularity and cultivation have all contributed to the wealth of genetic resources that have been developed associated with pea. This section reviews the current status and recent developments in pea genetic resources that are available within the public domain

A large number of *ex situ* germplasm collections for pea exist around the world (Table 4). Historically, these were established to provide access to a range of variation from the centres of diversity and different gene pools for taxonomic reference, research and to underpin breeding programs. These *ex situ* collections have a long history of active collaboration between each other and in supporting wider initiatives (Ambrose and Green, 1991). A working group for grain legumes exists as part of the European Cooperative Programme for Crop Genetic Resources which brings together the formal and informal sectors to collaborate on activities and initiatives of common interest such as the European central crop databases (ECP/GR). In the absence of a CGIAR institution with a global mandate for pea, an international consortium for pea genetic resources (PeaGRIC) has recently been formed that links together key collections within Europe, USA, ICARDA and

Australia. The aims of the consortium will be to coordinate pea genetic resources in the broadest sense and to provide stakeholder groups with a readily identified body with which they can interact. To this end two of the primary objectives of the consortium are to draw together key information resources and initiate the formation of a decentralised international core collection out of the many individual core collection initiatives.

Table 4. *Ex situ* germplasm collections of *Pisum* with holdings in excess of 1000 accessions.

FAO Institute code	Country	Number acces-sions	Web site for Germplasm searches
ATFC	Australia	6567	http://www2.dpi.qld.gov.au/extra/asp/AusPGRIS/
SAD	Bulgaria	2787	http://www.genebank.hit.bg/
ICAR-CAAS	China	3837	http://icgr.caas.net.cn/cgris_english.html
GAT	Germany	5336	http://fox-serv.ipk-gatersleben.de/
BAR	Italy	4297	http://www.ba.cnr.it/areagg34/germoplasma/2legbk.htm
CGN	The Netherlands	1008	http://www.cgn.wur.nl/pgr/
WTD	Poland	2899	http://www.ihar.edu.pl/gene_bank/
VIR	Russia	6790	http://www.vir.nw.ru/data/dbf.htm
ICARDA	Syria	6105	http://singer.grinfo.net/index.php?reqid=1151843332.3126
NGB	Sweden	2724	http://www.ngb.se/sesto/index.php?scp=ngb
JIC	UK	3194	http://www.jic.ac.uk/GERMPLAS/pisum/index.htm
USDA	USA	3710	http://www.ars-grin.gov/npgs/searchgrin.html

The development of core collections or test arrays that aim to represent a wide range of genetic variation within a restricted set of accessions with the least amount of repetition have been ongoing in a number of institutions for some years (Matthews and Ambrose, 1995; Swiêcicki et al., 2000; Coyne et al., 2005). The composition of these different initiatives varies with both the individual collection and the aims of the study. A core collection developed to represent the variation within cultivated material will differ considerably from those where the genus as a whole is the considered.

Interest in variants and mutant forms in pea has resulted in large collections of genetic stocks that is now extending to mapping populations and near isogenic lines. Early geneticists and breeders actively exchanged novel forms which over the years have coalesced into larger holdings. The first significant collection of such genetic stocks was formed by Herbert Lamprecht as part of his long career in pea genetics which spanned over 40 years (Blixt, 1963; Lamprecht, 1974). This collection was taken over by Stig Blixt, who further developed and expanded the work. He also went on to document and computerise the collection and actively promoted the use and utility of the underlying genetic information as a tool for breeding as well as research (Blixt and Williams, 1982). The collection became linked to the Pisum Genetics Association as the repository of seed on published mutants and their wild type counterparts. The long term future of these resources was further secured by the transfer of the active centre for this work to the John Innes Centre in the 1994. The collection has continued to develop with the same underlying aims and objectives

which is to collect, maintain and distribute genetic stocks and associated data for research, breeding and reference purposes. An online web searchable catalogue of the gene list with descriptions, images, reference germplasm and bibliography has now been developed (Ambrose, 1996; PGene).

A wide range of older heritage or heirloom material is maintained and in some cases selected by seed saver organisations (Seed Savers Exchange). These groups have sprung up in many countries and are good sources of diverse material and often have good working knowledge of their characteristics (Stickland, 2001, Irish Seed Savers Association). In addition, there are a number of good publications of detailed descriptions and illustrations of pea cultivars that offer useful reference information. As stated in section 3.1, these are often associated with one of a number of varietal classification systems current at that time (Hendrick, 1928; Mateo Box, 1955; Fourmont, 1956 and Sneddon and Squibbs, 1958). A noticeable point in comparing the cultivars described in these publications is just how wide spread the sources of this material and their general dispersal across Europe and north America.

From Mendel's seminal paper (1866), the use of pea as a model for inheritance and genetic studies has resulted in an extensive literature concerning cytology and genetics (Blixt, 1972). The somatic chromosome number of 14 was established by Cannon (1903). Studies of the pea karyotype and associated translocation points was extensively studied (Sansome, 1950; Lamm, 1951; Lamm and Miravalle, 1959; Folkeson, 1990). Lamprecht (1948) was the first author to present seven linkage groups claiming that they corresponded to the seven chromosomes of pea. The data available at the time was limited and inevitably, further work has led to extensive revisions to these original linkage groups and their chromosome assignments (Hall et al., 1997a and 1997b; Ellis and Poyser, 2002). The various genetic maps for *Pisum* are becoming increasingly well aligned as more markers are mapped and exchanged between mapping groups. The most recent map combines data from three different crosses and comprises of 239 microsatellite markers (Loridon et al., 2005) but other key maps that are of use include those of Lacou et al. (1998) and Weeden et al. (1998). A set of linkage maps that are particularly useful are three that were constructed between vining and combined peas (Ellis et al., 1992).

One of the problems still faced by breeders today is how to bridge the gap between broader genetic variation, whether in the form of exotic diversity or phenotypic variation represented in mutant collections and its availability in a form that can be easily used within breeding programs. With such a wide distributional range and long history of cultivation, the immense range of germplasm resources available for pea in *ex situ* collections represents an interesting paradox. They are considered of high value as resources which focuses around there containing important alleles and allelic combinations for the future of crop improvement, while at the same time our knowledge and understanding of the underlying structure and drivers of genetic variation remains limited (Ambrose et al., 2004). Investigations into the distribution of diversity within cultivated pea and their relationship to wilder forms have been performed for a number of reasons; to help understand and refine phylogenetic relationships within the genus, to help delineate differences between different cultivated forms (Amurrio et al., 1995) and in the structuring and management of germplasm collections. They have been used to explore the

relationships between different cultivated forms, the taxonomic structure and the organisation of germplasm collections and to assess the relationship between the different cultivated forms and wild germplasm. In recent years, the deployment of a range of molecular marker diversity studies in pea have had significant impact on the level of information that is available (Lu et al., 1996; Ellis et al., 1998; Pearse et al., 2000; Burstin et al., 2001; Vershinin, 2003; Baranger et al., 2004; Tar'an et al., 2005). The improved reliability of marker systems and the ability to develop them as high throughput systems (Flavell et al., 2003) means it is now realistic to consider the screening of whole germplasm collections. The first such example in pea is the application of retrotranspon element markers to the entire John Innes Pisum collection was commenced in 2000 (Flavell et al., 1998; TEGERM).

5 Major Breeding Achievements

The large range of cultivated forms available commercially by 1900 already represented a large primary cultivated gene pool. Already adapted to growing in a wide range of agroclimatic regions and with extensive variation for flowering time, plant habit and seed characters, pea breeders have had ample resources with which to work. Breeders have been consistently improving the pea crop without necessarily being able to define the genetic basis of what they have done. The majority of improvements in yield and performance of the crop have been through small incremental steps rather than large ones. While the geneticist is often a reductionist, dissecting pathways down to individual components, breeding is about the integration of a complex range of inputs and variables whose interactions are mostly poorly understood. Small wonder then that the commercial pressure of breeding results in the majority of the effort going into crosses between mostly elite material.

There are nevertheless some definable developments from recent decades that are worthy of note. A major problem associated with the pea crop is its tendency to lodge or its lack of standing ability. The pea plant is a natural scrambler and its long vines and tendrils make it ideally suited to growing through other vegetation. One of the traditional ways of growing peas it against support either in the form of small branches or twigs or against wires. Grown as a monoculture the planting density is such that neighbouring plants become attached to each other within the canopy, while this may keep the crop standing for some time, the canopy, in a good number of cases collapses with the weight of pods and seeds as the crop matures. This greatly impedes harvesting and creates an ideal micro-climate for fungal diseases. A major contribution to combating lodging was the incorporation of the recessive allele of the afila gene (*af)* that converts leaflets to tendrils (Fig 2 a and b) in the 1970s leading to the development of the 'semi-leafless' pea (Snoad, 1974; Davies, 1977; Hedley and Ambrose, 1981). The presence of additional tendrils that interlock with each other help make a more rigid upper canopy, while also allowing more light and air circulation deeper into the canopy. Since the release of the first cultivars carrying this trait it has been used in breeding programs worldwide and a majority of new cultivars carry this trait. The resurfacing of an induced afila allele expressing an

intermediate form bearing a pair of leaflets in addition to the tendrils (Fig 2 c.) offers further possibilities for breeders to explore (Ambrose, 2004).

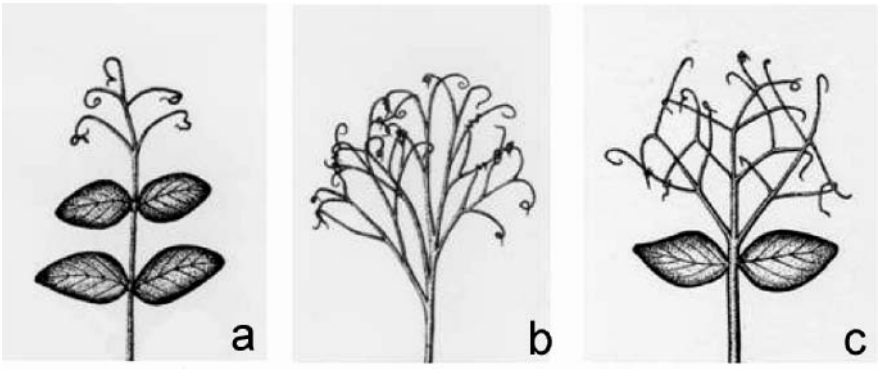

Fig. 2. Phenotypes associated with alleles of the alfila (af) locus. a. wild type AfAf, b. afaf, c. afaf$^{11/47}$.

Significant advances in the incorporation of disease resistance into modern cultivars is now becoming more routine and thus helps to reduce crop inputs. One notable example is resistance to powdery mildew (*Erysiphe pisi*) reported by Harland (1948) which still confers good resistance today and shows no sign of breaking down. The incorporation of resistance or tolerance to a range of viruses and a range of fungal diseases is becoming more standard.

The strategy with the commercial vining pea crop has been to maximise the proportion of developing embyos of desired size at the right stage of development. This has been approached using a number of strategies including selecting for high number of flowers per node (Hardwick et al., 1979), trying to develop a more determinate plant habit with a restricted the number of flowering nodes and by selecting for simultaneous flowering at multiple nodes (Marx, 1977), more ovules per pod providing more embryos at the required stage. Fasciation which result in an increase in the number of flowers borne in the apical region of the plant (Fig. 3) have been tested as an alternative means to achieving a higher proportion of embryos at the required stage of development (Gottschalk, 1977). This character exists in older long vined picking varieties and has come in and out of favour with breeders over the years as there is also a tendency in wet seasons for falling petals to become lodged in leaf axils and offer sites for botrytis and other pathogens to invade. A number of new varieties can be found described as semi-fasciated (fascinated but low to medium expression) within pea trials in the UK. While genetic variation exists for all these traits, the translation of their potential into real physiological gains within the crop has been slow. The physiological load of the developing seeds on the plants competing in the crop environment is a complex one to model (Marx, 1977) and in commercial plant breeding, the opportunities to develop different plant ideotypes to the point where they can be tested against each other is a rare event. The only trait of these that has successfully been exploited is that known as multipod where 3-4 pods are successfully held on a raceme but this type represents only a small fraction of

varieties and is not universally successful or reproducible across sites and years. It can only be hoped that opportunities to engineer further changes to the plant architecture will emerge.

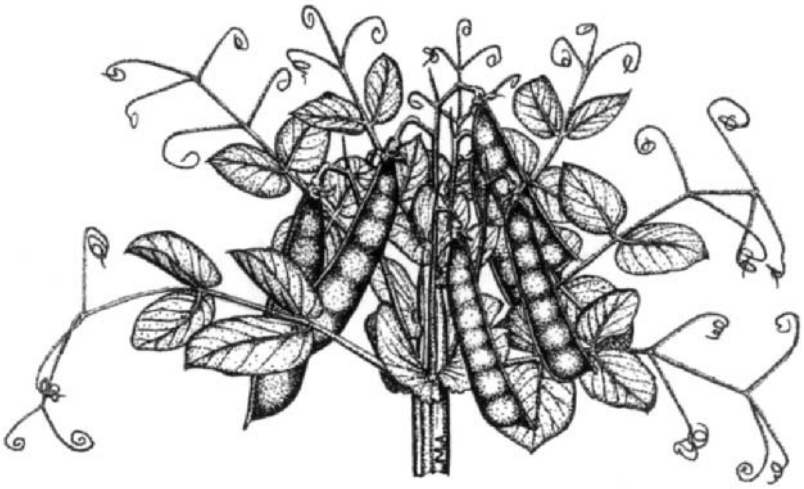

Fig. 3. Apical fasciation in cultivated pea.

6 Current Breeding Goals

The overarching goals of breeders will always be yield, quality and consistency. Dissecting these into their components traits requires a constant review of new knowledge and resources with a view to their application or incorporation into crossing programs. The ever present challenges of biotic and abiotic stresses are also a high priority for action and the changing weather patterns being experienced in many regions of the world only increases the degree of difficulty and crop management required in dealing with the crop. Having said that, breeders have consistently delivered new plant varieties that outperform earlier types. It is interesting to note however, that some older commercial lines, for whatever reason, continue to be popular and remain in cultivation. Having already developed a range of successful forms for the various market types which have come through the system, breeders can be confident that the basic plant models are fit for purpose.

In terms of plant architecture the overriding problem associated with the pea crop is still its variable standing ability. The character is frequently included in varietal assessments and the introduction of the semi-leafless form referred to in section 5.1, has certainly helped considerably, lodging remains a problem even in relatively short

strawed types. Descriptions of stiffer stemmed forms can be found in the literature but have proved disappointing. Studies into the mechanics of stem strength have not contributed anything tangible or consistent to date. Their poor description and understanding however suggests this is an area that might warrant revisiting. Fasciation and its multiple role in broadening the upper sections of the stem, synchronising flowering and clustering the pods at the top of the canopy rather than being spread throughout, is still being used by breeders who are able to select forms with moderate degree of expression hence the emerging use of the term semi-fasciated type.

The pea crop still suffers from a wide range of pests and diseases (Kraft and Kaiser, 1993; Kraft and Pfleger, 2001). Resistance or tolerance to pests and diseases, while readily taken up by some breeders are becoming more frequently used as the reliance and costs of agrochemical controls on vegetable forms becomes a more contentious issues with respect to consumers and their impact on the environment. Good sources of resistance to many pea diseases have been documented (Hagedorn, 1984; Lewis and Matthews, 1984; Ali et al., 1994). The highest priority disease targets today centre on foot and root rots, especially *Aphanomyces euteiches,* downy mildew (*Peronospora viciae*) and pea blight (a complex of species including *Ascochyta pisi, Mycospaerella pinodes* var. *pinodella,* and *Phoma medicaginis*). Aphanomyces root rot has become one of the most destructive pea diseases worldwide. Tolerance first reported by Marx and colleagues (1972) proved unusable due to tight linkage to alleles that adversely affected the vegetable product. Kraft successfully recovered partial resistance in breeding lines with desirable horticultural traits in 1988. With the emergence of new strain and short rotations between pea crops the problem increased and with no effective fungicide treatment, efforts to find additional sources of resistance has been the subject of large-scale international collaboration to screen germplasm for new sources of resistance and incorporate sources of partial resistance into breeding programs. A number of QTL's for resistance, one that appears consistent over years and across sites and four minor ones were reported by Pilet-Nayel et al., 2002. Markers are now being developed to assist with the selection of resistant progeny for breeders. Downy mildew is present in many pea growing areas but is only of economic importance in regions which experience high temperatures and humidity and even then its severity depends on the timing of infection (Kraft and Pfleger, 1993). Resistance was reported by Matthews and Dow (1976), but recent results suggest this resistance is breaking down (Thomas et al., 1999). A renewal of effort between pathologists and breeders is therefore required to deal with this disease. *Ascochyta pisi* causes leaf and pod spot and can cause serious blemishing in vining peas. Sources of resistance and host differentials are available for all the causative species that form the Ascocyta complex but the complexity of the disease when encountered in the field and the multigenic nature has lead to slow progress in the utilisation of the available sources of biological resistance. High priority target pests of pea include aphids (*Acyrthosiphon pisum*) and bruchids (*Bruchus pisorum, B. affinis*). Large infestations with aphids can cause stunting of the plant and damage to foliage and pods by their feeding. These often occur as temperatures rise and there is a concomitant problem with drought stress under which the symptoms may become even more severe. They also act as a vector in the transmission of some 30

plant viruses. Bruchids are a serious problem in growing regions where they occur feeding on pollen and then go on to cause damage to the developing seed where the larvae feed and continue their development thus rendering the seed unmarketable. There are no reports of resistance to bruchids in cultivated sativum germplasm although resistance has been reported in studies from the secondary genepool of *Pisum fulvum* (Hardie, 1990; Clement et al., 2002). Recent work has also established an inducible resistance conditioned by the recessive allele for neoplastic pods (*np*), where neoplastic outgrowths of undifferentiated tumour-like cells develop in response to oviposition on developing pods which impede larval entry into the pod (Doss et al., 2000). The possibility of combining these findings offers further potential advances in increasing the plant defences against this pest.

Quality traits and post harvest changes represent the final category of challenges to breeders. Having developed material that germinates, grows and survives to be harvested, maintaining the quality of the product to the consumer poses technical as well as biological problems if premium prices are to be achieved. One way of approaching this is to seek to minimise the wastage of what is harvested by seeking more uniformity of the developmental stage of the seed or pods, or by seeking small incremental steps that can be achieved by incorporating traits resulting in peas retaining a darker colour and not becoming bleached. At present, little is known of the complex metabolite profile of what makes a good vegetable pea and which compounds cause problems. New techniques to explore these questions are now being tested but currently, these complex issues are poorly understood. The produce delivered by breeders and growers across the market types is already a good one and any progress made is more likely to be as a result of closer cooperation and operational management of the supply chain.

7 Breeding Methods and Techniques

Pea behaves and is managed as an inbreeding crop. There is a low rate of outcrossing estimated at below 1% (Gritton, 1980) although this figure may rise under stressed conditions where flowers are generally smaller and the stigmatic surface protrudes through the keel. As an inbreeder, the most widely practiced breeding method is the pedigree breeding system through transgressive segregation from crosses. Selection for high heritability traits is frequently practised in the early generations before lines are grown out as small plots around the F_4 generation. Some breeders employ the strategy of growing and selecting alternate generations (F_3 and F_5) at off-site locations in countries in the opposite hemisphere immediately following harvest in their target region to reduce generation time. There is widespread use of the single seed descent system utilising glasshouses or plant growth rooms to speed up early generations while also maintaining a wider level of variability between lines before growing out small micro-plots of plant progenies for field evaluation and selection. Bulk selection is also used by some breeders where the F_2 is grown as a bulk which is split into smaller units, a portion of which are grown in small plots while others are grown as individual plants to facilitate selection based on plot performance. Recurrent backcross and selection is commonly used to introduce single desired trait

such as disease resistance or quality trait from less adapted material into elite backgrounds. An example of this is the development of lines carrying resistance to pea seed-borne mosaic virus (PsBMV) in eight varietal backgrounds (Muehlbauer, 1983).

There is a long history of mutation breeding in pea where chemical or radioactive mutagenic agents are deployed on commercially proven cultivars of the day to induce random mutations across the genome some of which may prove useful in crop improvement such as stem thickness or simultaneous flowering but many more may be of potential use in breeding (Blixt et al., 1991). The method was widely adopted in the 1960's and 70's in Sweden (Lamprecht, 1974), Italy (Saccardo et al., 1986), Germany (Gottschalk, 1977; Gottschalk and Wolff, 1977), Poland (Jaranowski and Mickle, 1985) and Bulgaria (Vassiliva, 1978). All are examples of collaborations between research geneticists and breeding programs, mostly in the public sector. The method has proved of mixed fortunes in that it has undoubtedly resulted in many novel alleles at both known and new loci which have contributed very significantly to research into the understanding of basic plant development and to the pool of variation that is available for breeding, while in practical breeding terms, few induced mutations have found their way into finished varieties to date. The first registered variety was of Stral-ärt in 1954 (Gelin, 1955) with a few others following in the late 1970's to mid 1980's (Jaranowski and Mickle, 1985; Saccardo et al., 1985). None have been reported in the intervening period. In some respects this is a rather harsh criticism, as induced mutations should be taken in a more collective context of the use of mutations in the broad sense. On this basis it is clear that mutants have provided a significant range of variation that is widely represented in modern breeding material. The frequency of uptake of any single trait into breeding is extremely low in both forms but they do occur. It is important therefore that they remain available and re-evaluated over time to ensure earlier efforts of inclusion do not prejudice future gains. Each generation of breeders and researchers needs to become acquainted with this wider variation as they are represent some of our most readily available resources for crop development. A wide range of mutations isolated from past programs, have been incorporated into public germplasm collections, the majority are still available and studied today (Blixt, 1972; Swiêcicki et al., 1981; PGene). Each generation of breeders and researchers needs to become acquainted with this wider variation as they are represent some of our most readily available resources for crop development.

While there are no examples of mutation breeding being undertaken today, there are numerous mutagenesis programs to be found in public sector research. These programme underpin basic research and resource development in plant developmental genetics and genomics. The regular dialogue and collaboration between the research and breeding communities means that any new allelic variation of potential agronomic use are generally readily available to breeders and joint development of material is also not uncommon (GLIP). Recent examples of induced chemical mutations of interest to breeders include new genes regulating basal branching (Rameau et al., 1998) and bulbous base (*blb*) which results in a thickening of the hypocotyl region at the base of the stem (Kosterin and Rozov, 1993) which may help in strengthening the stem base which is naturally weak. Alleles at the *rug3* locus

encoding the enzyme plastidial phosphoglucomutase which is involved in the starch biosynthetic pathway, have resulted in a patented method for increasing sucrose content of plants (Harrison et al., 2000). This gene is of potential value in breeding for the frozen pea market as its regulation results not only elevated levels of sucrose compared to conventional wrinkled high sucrose forms, but sucrose levels remain elevated over a longer period of seed development thus offering an extended harvesting window for the crop (HU9903062 1999).

Apart from isolated instances of close linkage between morphological characters, The identification of linked markers to genes of interest for use in marker assisted selection (MAS) within breeding programs started with the mapping of isozyme markers (Weeden and Marx, 1984). The prime targets for MAS are traits that express late in plant development. This allows for the early screening of large populations of seedlings, which would be costly to grow on to screen later on. Such targets include disease and lodging resistance and seed characters. The first isozyme marker linked to a resistance gene was that of alcohol dehydrogenase (Adh-1) linked to resistance to pea enation mosaic virus (*En*) and was reported by Weeden and Provvidenti (1987). The rapid developments in marker systems over recent years, their increasing ease of application and lower unit costs has resulted in breeders becoming more interested in deploying MAS as they become available. Two recent examples related to disease resistance are the development of PCR markers designed from cDNA-AFLP fragments providing tight linkage to genes (*sbm*-1, *mo*) conferring resistance to pea seed borne mosaic virus (Gao et al., 2004) and an SSR marker suitable for MAS for powdery mildew resistance in pea (Ek et al., 2005). The recent development of a simple marker system developed to enable the identification and selection of progeny with low levels of seed trypsin inhibitors is an example of the type of resources that are increasing as a result of the more detailed genetic maps (Page et al., 2003). A further example that highlights a future development involves the identification of quantitative trait loci (QTLs) for lodging resistance using two markers that successfully led to the identification of F2:3 families with significantly lower lodging scores (Tar'an et al., 2003: Warkentin et al., 2004).

8 Integration of New Biotechnologies in Breeding Programs

The use of new technologies in breeding vegetables peas is restricted to the frozen and canning sectors which are the only sectors that generate sufficient revenue to invest in such operations and even here, the R&D spends are not large. As stated previously, there are a number of examples of vegetable and combined pea breeding both employ marker assisted selection and mapping capacity although this number is still limited. This section presents examples of other technologies that have or are still being used in breeding programs but the individual efforts remain focused and relatively small. It is true to say that the combined commercial breeding sector in pea is insufficient to sustain the development of such technologies itself and relies on public sector and consortium arrangements to help develop and sustain innovation in these areas. Their contribution is often by way of material, expertise and other in kind contributions. The main regulators that restrict the uptake of new technologies

in breeding programs are consistency and cost per data point or sample. As reproducibility both within and across groups and platforms improves and the cost per assay or run comes down, the more likely it is that the utilisation of such technologies alongside breeding programs will become more widespread.

Transformation and regeneration protocols have been available for pea for some time and are regularly used in research work. The most widely adopted method for pea is that of *Agrobacterium tumefaciens* mediated transformation (Puonti-Kaerlas et al., 1990; Schroeder et al., 1993; Bean et al., 1997). Difficulties arise from the fact that only a small proportion of cultivars prove suitable for either regeneration or transformation and the basis of this variability is not understood. Having said that, a sufficient range of cultivars have been found suitable for the work to not be unduly limited by this factor. Somoclonal variation arising from the regeneration of plants from callus led to the use of cotyledonary meristems from freshly imbibed seed as a source of tissue for transformation (Bean et al., 1997). Improved strains of *Agrobacterium* have only resulted in slight improvements in performance and overall transformation efficiencies are still only around 4% at best. Nevertheless, the use of these procedures in research ensures that capacity in this field is being maintained. The use of these technologies in breeding is limited to proof of concept. One example is the partial resistance to alfalfa mosaic virus (AMV) gained as a result of transformation with a chimeric virus coat protein gene (Grant et al., 1998). A second example is the transfer of α-amylase inhibitor (α -A1) and the promoter phytohemagglutin, both found in *Phaseolus vulgaris* and were shown, when constitutively expressed in pea, to confer resistance to pea weevil (*Bruchus pisorum*) (Schroeder et al., 1995). The expression of the inhibitor served to block the development of the larvae at an early stage in development and so seed damage was minimal and seed quality much improved. Legal complications over some of the technology used effectively stopped this work at the early stage of field testing. The transfer of herbicide resistance both as a reportable marker and a trait to benefit the crop have been reported but not carried through to commercial release. While GM crops are on the increase in many parts of the world, the adverse reaction to GM crops in Europe and the low rates of transformation and target genes to transfer have all contributed to the pea breeding industry not engaging in the development of GM peas to date. In fact, in contrast to soya, peas can be frequently found to be promoted using their non GM status as a positive marketing strategy. No doubt this situation will change over time as further refinements to the technologies and good candidates for gene transfer emerge to produce real benefits for the grower and consumer. A recent improvement to the process has been the development of so called ‘clean gene technology’ whereby vectors are formed that carry two T-DNAs, the first carries the gene of interest and the second the selectable marker. In some cases the two genes insert into unlinked locations and thus plants containing only the gene of interest can be identified among the progeny.

The every growing array of technologies and genomic tools that are being developed for model species are already starting to impact on breeding of a number of crop species. For pea the model legume species are *Lotus japonicus* and *Medicago truncatula*. The developments include platform technologies and resources that bridge the gap between functional and structural genomics and link their discovery in

models to application in crops (Waugh et al., 2006). Genotype assisted breeding has recently emerged as an approach to marshalling functional markers and informatics that in principle will allow *in silico* design and selection. A big challenge that these approaches present is the requirement for detailed phenotypic data of agronomic and processing traits. While the costs remain relatively high for inbreeding species and especially where they represent only a minor crop, these will come down in time and MAS and selection will gradually evolve into genomics assisted breeding (Varshney et al., 2005). A clear mobilisation of the legume community to address these concerns and develop a combined efforts between researchers and breeders is reflected in a number of ongoing multidisciplinary programs. One example is the pulse crop improvement network (PCGIN) in the UK which brings together legume research community, breeders and processors where correlating genotype to phenotype is one of the prime objectives along with the development of germplasm and genomic resources for use in the applied sectors. The development of forward and reverse genetic platforms are further examples of new tools that are of potential benefit to breeders. Two complementary reverse genetics tools developed as part of the European grain legume integrated programme (GLIP) are now operational. The first is a pea tilling platform (PETILL) based on two EMS mutagenised populations and the rapid systematic identification of mutations in target sequences. The second is allele mining or EcoTILLING which is based on the analysis of natural allelic variation for the gene of interest in sets of germplasm. Both are high throughput systems that represent an effective way to generate or identify variant alleles in specific genes of interest.

The rapid developments in comparative mapping and sequencing across legume species is set to greatly benefit pea breeding through access to a wide range of tools and resources. The high degree of co-linearity between the *Pisum* and *Medicago truncatula* (Kalo et al., 2004), genetic maps and the large international sequencing effort in *Medicago* and *Lotus japonicus* brings an unprecedented range of tools, resources and data into the compass of those researching and breeding pea.

One of the current priority areas for legume research is in exploring the complex interactions with micro-organisms, particularly, arbuscular microrrhiza and nitrogen fixing bacteria. One of the primary features that pea, along with many other legume species exhibit, is their ability to fix atmospheric nitrogen through the complex and elegant process of symbiotic nitrogen fixation in association with *Rhizobium leguminosarum* (Brewin et al., 1993). This process enables peas to grow well on poorer soils and agronomic manuals state no N-fertilizer is required. Exploration of the efficiency of plant genotypes, bacterial strain and their interaction have highlighted significant effects and highly heritable variation (Hobbs and Mahon, 1982; Skøt, 1983. The understanding and manipulation of these processes has been a key area of research over many decades. The *sym2* gene associated with race specificity identified in peas from Afghanistan (Lie, 1978) and subsequently found to be widespread in ecotypes and landraces from the eastern mediterraneum, Turkey and Iran (Young and Matthews, 1982), opened up the possibility of linking this specificity to 'improved' strains of *Rhizobium* that could be applied as inoculum to the crop in an effort to improve the nitrogen fixation capacity of the crop. The inability of the introduced strains to compete with the natural *Rhizobium* strains led

to this approach being abandoned. With the exception of inoculation of soils in regions where peas have not been previously grown, there has been no demonstrable improvement in nitrogen fixation that has arisen out of this research to date. In general terms the rhizosphere is an area where breeders are naturally reluctant to engage with on the principle that, with the exception of specific problems associated with mechanical and mineral composition, good performance of the crop indicates that things are generally going well. While they may not be maximising the nitrogen fixation efficiency of the crop, it is not one of their higher priorities in that the resources needed to engage in seeking improvements look unlikely to be matched by the returns. It is generally thought that looking for plants that show active fixing nodules under conditions of moderate soil N fertility is desirable and relatively easy to assess. Undoubtedly, this very complex system which will require more interdisciplinary research to unravel the processes before improvements and usable approached to screening in the field situation can be made (Provorov and Tikhonovich, 2003).

9 Seed Production

Peas are an inbreeding crop and so no special isolation practices are required. Multiplication rates per plant vary greatly depending on flowering time of the line and seed size and can be anything from 20-300 seeds. Multiplication rates in the field can be increased by lowering sowing density or by growing plants at 10 cm spacing against wire to act as support with 1m between rows. Grown against wire, multiplication rates of 50 to 100 fold can be expected. Grown as single plants in pots, the multiplication rate can be increased by pinching out every other flower to spread the sink demand. The vigour of some wrinkled seeded varieties, is known to be affected when sown into the adverse conditions of cold and wet soils. This pre-emergence failure is understood to result from imbibition damage due, in large part, to cracked seed coats sustained in the previous generation when seed was harvested at seed moisture content of 10-15% (Biddle, 1980; Matthews et al., 1980).

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Snap Bean

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1 Introduction

The common bean (*Phaseolus vulgaris* L.; 2n = 2x = 22) is a member of the family Fabaceae, tribe Phaseoleae, subfamily Papilionoideae. Cultivated forms are grown on all continents except Antarctica (Gepts, 1998). Commonly grown species of *Phaseolus* are: *P. acutifolius* A. Gray (tepary bean), *P. coccineus* L. (scarlet or runner bean), *P. lunatus* L. (Burma, butter or Lima bean), and *P. vulgaris* L. (baked, canellini, common, dwarf, flageolet, frijoles, French, kidney, navy, pinto, snap, string, wax, haricot or Nunas bean) (Broughton et al., 2003). Beans main products are dry beans (seeds harvested at complete maturity), shell beans (seeds harvested at physiological maturity, i.e. before the desiccation associated with complete maturity sets in), and green or snap beans (pods harvested before the seed development phase) (Gepts, 1998).

Wild beans grow in a wide arc stretching from northern Mexico (approx. 30^{o}N) to Northwestern Argentina (approx. 35^{o}S), at altitudes ranging from 500 to 2000 m and rainfall from 500 to 1800 mm (Gepts, 1998). The production of cultivated bean spans from 52^{o}N to 32^{o}S latitude (Schoonhoven and Voysest, 1991), from sea level in the continental USA and Europe to elevations of more than 3000 m in Andean southern America (Graham and Ranalli, 1997). Common bean possesses the maximum breadth of adaptation of all *Phaseolus* species, which are extremely diverse crops in terms of cultivation methods and morphological variability. Among *Phaseolus* species, common bean is the most widely grown, occupying more than 85% of production area sown to all *Phaseolus* species in the world. Many varieties of beans achieve high yield over a wide range of environments (Singh, 1992).

There are two major commercial classes of common bean varieties, snap and dry beans. Snap bean varieties possess a thick succulent mesocarp and reduced or no fibre in green pod walls and sutures (Silbernagel, 1986). The green pods are harvested for fresh, frozen and canning purposes. Among snap bean varieties, there

can be a large variation in growth habit and adaptation traits (Singh, 2001). Different market classes of snap bean varieties are largely determined on the basis of pod shape (flat, round or oval), colour (dark green, light green, yellow or purple), and length or sieve size. Similarly, large variation in growth habit, phenological traits, seed size, shape, colour, and canning and cooking qualities are found among dry bean varieties (Voysest and Dessert, 1991; Singh, 1992). The largest production (>14 million hectares) and consumption of *P. vulgaris* in the world is of dry beans, followed by a much lower production of snap beans (Singh, 2001).

The snap bean varieties have developed since the last quarter of the nineteenth century, and especially during the last 50 years in Europe and the USA. At least 200 commercial varieties are known and many local varieties with inferior quality and greater degree of horizontal disease resistance are grown (Grubben, 1977). Snap beans belong to the determinate type, with a pod production in as little as two months, or to the indeterminate type, which are continually harvested for about six months (Broughton et al., 2003). However, both temperature and photoperiod have strong effects on crop growth and development (Masaya and White, 1991). These effects often exert a primary influence on the selection of varieties and planting dates at a given site. The snap bean types appear to have less climatic adaptation than the dry bean types (Rubatzky and Yamaguchi, 1997). Also, pole snap beans tend to grow better at slightly cooler temperatures and are more sensitive to high temperatures at flowering than bush types (Rubatzky and Yamaguchi, 1997).

The genus *Phaseolus* is a valued grain legume or pulse crop (Evans, 1976). Legumes are vital in agriculture as they form associations with bacteria that fix nitrogen from the air. Effectively this amount of internal fertilization is the main reason for which legumes are richer in proteins than all other plants. Thousands of legume species exist but common beans are the most important grain legumes for direct human consumption in the world. They provide a primary source of dietary proteins, with bean seeds containing between 20 and 25% proteins, and carbohydrates, as well as other minerals, such as iron (Lott et al., 2000). The main storage protein is phaseolin and is deficient in sulphur-containing amino acids, principally methionine. This deficit is made up by including cereal seed storage proteins in the diet, which are themselves deficient in lysine (Broughton et al., 2003). Snap bean has similar nutritional value, when compared to dry bean on a similar moisture basis (Shellie-Desert and Bliss, 1991). Green pods are a superior source of calcium, iron and vitamin C (Grubben, 1977).

According to data published by the Food and Agricultural Organization (FAO, 2006) for snap beans, the world production in 2006 was 6333047 t, of which Asia produced 72.3 percent, Europe 15.5 percent, Africa 8 percent, North America 3.3 percent and Central America 0.9 percent. Among the leading producers were (in decreasing order) China, Indonesia, Turkey, India, Egypt, Morocco, Spain, Italy, Belgium, France, and USA (FAO, 2006).

2 Origin and Domestication

2.1 *Phaseolus* Origin

The genus *Phaseolus* is native to the Neotropical region of America (Broughton et al., 2003). Wild *P. vulgaris* had already diverged into two major gene pools, each with its characteristic geographic distribution in Mesoamerica and the Andes (Gepts, 1998). These two wild gene pools can be distinguished at the morphological (Gepts and Debouck, 1991) and molecular level (Becerra-Velasquez and Gepts, 1994). Five species of *Phaseolus* were domesticated: *P. coccineus*, in Mexico; *P. vulgaris*, in both Meso- and South America, probably from at least two independent domestications (Gepts, 1990); *P. lunatus*, which also appeared to have been independently domesticated in Mexico and Peru (Gepts, 1990); *P. acutifolius* A. Gray, in Mexico (Evans, 1976); and *P. polyanthus* Greenman (Debouck, 1999; Singh, 2001). The wild progenies of all five domesticates are known and their remains have been dated back to 10700 BP (Evans, 1993). Their pods dehisce along both sutures, and their seeds are smaller and more slowly permeable than those of the domesticated (Kaplan, 1965).

A reasonable estimate of *Phaseolus* species would be 50-60 species, pending additional germplasm explorations in Central America (Debouck, 2000). Recent phylogenetic studies that included both wild and domesticated species of *Phaseolus* using morphological, biochemical and molecular data have confirmed that the genus is monophyletic (Debouck, 1999). Studies on cpDNA (Delgado-Salinas et al., 1993) and intergenic transcribed sequences (ITS) (Delgado-Salinas et al., 1999) have established a phylogeny for the entire genus. Each cultivated species forms a primary gene pool with its wild ancestral form (Smartt, Debouck, 1999). Secondary and tertiary gene pools may exist for all of them, depending on phylogenetic events that lead to the formation of the biological species, long before domestication took place (Debouck and Smartt, 1995). Molecular data have thus confirmed that all these species can be intercrossed, although the degree of difficulty and the viability of reciprocal crosses vary (Hucl and Scoles, 1985; Waines et al., 1988). Remarkable diversity of morphology occurs within this group of species (bushes to climbers, seed colour and colour patterns), adaptation (from hot deserts to cool mountain environments), and reproductive systems (from cleistogamy to out-crossing) (Broughton et al., 2003).

As indicated, wild beans dispersed both northwards and southwards to form two geographically distinct gene pools in Mesoamerica and the southern Andes (Gepts, 1998). These geographically distinct gene pools qualify as sub-species based on the existence of partial reproductive isolation between them. Pairs of complementary genes that influence either the F_1 (dominant alleles) or later generations (recessive alleles) are genetically responsible for the isolation (Gepts and Bliss, 1985; Singh and Molina, 1996). Preliminary estimates show a divergence time of some 500000 years between these two gene pools (Coulibaly, Broughton et al., 2003). Thus, *P. vulgaris* is, at this stage, unique among crops in that two evolutionary lineages tracing back to the same ancestral populations that have been identified (Broughton et al., 2003).

Dispersal from the Andean and Mesoamerican centres of origin appears to have followed different routes. Gepts (1988) suggests that the smaller seeded Mesoamerican lines followed a route through Mexico and Central America, via the Caribbean and northern South America to Brazil. Common bean remains found in the Southwestern USA are also likely to have been introduced from Mesoamerica (Kaplan, 1965). Paredes and Gepts (1995) report extensive introgression of middle-American germplasm into Chile. In North America beans spread through California (5000 to 2000 B.P.) (Evans, 1976). In post-Columbian times, Spanish galleons took beans across Pacific to the Philippines and to Asia, also from Peru to Madagascar (Westphal, Evans, 1976). The slave trade took the beans from Brazil to Africa and subsequently inland to the trade routes. The other route followed by the majority of the varieties found in Europe, which are the larger-seeded Andean type, probably reached Europe via the Iberian Peninsula in the early 16th century, in 1534 for *P. vulgaris* and 1550 for *P. coccineus* (Schachl, 1998). In Europe the snap beans spread rapidly in the 16th and 17th centuries, and reached England by 1594 (Purseglove, Evans 1976), and Germany (Schachl, 1998). After 1500 *Phaseolus* spread eastwards over the Mediterranean basin of Europe and into middle Europe. Beans were cultivated all over Italy, Greece, Turkey and Iran in the 17th century (Evans, 1976). In the 17th and 18th centuries the bean reached the East African gene centres, through Arab traders (Schachl, 1998). In the late 19th century, in the eastern part of the USA there were numerous introductions from Europe (Evans, 1976) via immigration (Graham and Ranalli, 1997).

2.2 *Phaseolus* Domestication

The very broad distribution of wild beans raised several questions about the number of domestication events, the levels of genetic diversity in wild and cultivated forms, and the genetic divergence between wild and cultivated beans (Gepts, 1998). More recent studies of the role of gene flow between wild and cultivated beans have gained prominence, as have studies on patterns of genetic diversity and pathogenicity of related organisms. The common bean was domesticated in the upland regions of Latin America more than 7000 years ago (Gepts and Debouk, 1991). The domestication history of the common bean is well known and has been reviewed by Koinange et al. (1996). Wild progenitor and cultivated descendants generally give viable and fertile progeny and display contrasting differences for many traits constituting the crop domestication syndrome (Koinange et al., 1996).

Many evolutionary changes have occurred in *Phaseolus*. Changes as the determinate bush habit and low fibber content probably originated in mutations, that ancient civilizations were able to recognize, perpetuate and utilize (Silbernagel, 1986; Debouck, 2000). The growth habit is characterized by a combination of traits comprising determinacy, non-twining branches, few vegetative nodes, and long internodes (Silbernagel, 1986). The seed dispersal mechanisms were undesirable and economically unacceptable. In *Phaseolus* beans the lack of pod dehiscence is due to the combination of two characters, reduction of parchment layer and absence of fibbers along the two pod sutures (Debouck, 2000). Both characteristics are under the control of a few major genes (Bassett, 1976). The stringless character and the

round pod shape were incorporated into breeding programs within the past 100 years (Silbernagel, 1986). Common bean may have been domesticated first as snap bean because before ceramic times little was known about detoxifying antinutritional factors (Debouck, 1991). Selection of another character, as of daylength-insensitive varieties, may have already been started in central Chile by Araucanians (Wilhelm de Mosbach, Debouck, 2000). The short-day photoperiod requirement with the dwarf bush growth habit is believed to permit bean culture to higher latitudes (Silbernagel, 1986; Debouck, 2000). The growth habit genes are linked in coupling to the allele for photoperiod insensitivity (Coyne, 1967; Koinange et al., 1996), and may also be linked to other genes affecting the degree of climbing ability (Gepts, 1998). A recent selection in common bean is for large pod "gigantism" (Westphal, Debouck, 2000), which shows different or no anthocyanin pigmentation (Silbernagel, 1986).

Pod structure has been altered in cultivation with the reduction in dehiscence and fibre content. Three distinct pod textures are found in the common bean: the parchmented types which are very fibrous and dehisce strongly at maturity; the leathery types which are less dehiscent but split readily along the sutures; and the fleshy or stringless types which are indehiscent and do not split readily. Varieties with parchmented pods are used only for dry seed production; leathery podded varieties can be used for green pod production when young, or haricot production when fully mature; fleshy podded or stringless types are used entirely for the production of green pods (Smartt, Evans, 1976). A type of dormancy, hard-seededness, is another wild trait that the domestic varieties do not have (Silbernagel, 1986).

Genetic control of the domestication syndrome involves genes that have a major effect and account for most of the variation observed (>60%). As domestication of the common bean probably proceeded rapidly, adaptation to rapidly changing environmental conditions must have involved genes with major phenotypic effects (Koinange et al., 1996). The major genes or quantitative trait loci (QTL) that influence the domestication syndrome have been identified and mapped (Koinange et al., 1996; Gepts, 1999).

3 Varietal Groups

Beans are classified primarily for commercial reasons and to facilitate household and scientific communication. In general, common bean varieties can be grouped according to many criteria, the most important being those related to marketing and agronomic characteristics (Voysest and Dessert, 1991). Fewer and less distinct market groups of snap bean exist compared to dry beans (Myers and Baggett, 1999), but given the importance as a vegetable, breeders have developed many varieties, which can be categorized by plant traits and utilization or mode of consumption.

3.1 Varietal Grouping by Plant Traits

3.1.1 Varietal Grouping by Growth Habit and Plant Architecture

Growth habit and plant architecture in snap beans fall into a range similar to that found in dry beans, and are of primary importance in describing snap bean varieties.

According to a morphoagronomic concept, growth habit is defined as the result of the interaction of type of growth of stem and branches, number of nodes on stem, length of internodes, climbing ability, and branching pattern, that determine plant architecture (Hidalgo, 1991). Variation in growth habit appears to be continuous from determinate bush to indeterminate, extreme climbing types (Singh, 1999). However, for simplicity, agronomic value, and because of their adaptation to different cropping systems, Singh (Singh, 1999) used the type of terminal bud, stem strength, climbing ability, and fruiting patterns to classify growth habits into four major classes. These are: type I = determinate upright or bush, type II = indeterminate upright bush, type III = indeterminate, prostrate, nonclimbing or viny semiclimbing, and type IV = indeterminate, strong climbers. Generic or common classification often divides beans into two or three groups: bush and climbing beans; or bush, semiclimbing, and climbing beans. Dwarf, runner (or half-runner), and pole beans are synonymous to the above, although sometimes runner bean also refers to *P. coccineus* (Voysest and Dessert, 1991).

3.1.1.1 Determinate Bush Habits (Growth Habit I). Although several classifications include only one type in this group, it seems that at least two groups can be recognized here (Evans, Debouck, 1991): (i) the few-nodded bush or dwarf type (3-7 trifoliate leaves on the main stem before the terminal double raceme), that comprises materials selected for earliness in Europe and the United States; and (ii) the many-nodded type (7-15 or 15-25 trifoliate leaves on the main stem of Middle American or Andean origin, respectively), with some climbing ability. Most varieties, especially for fresh market and processing, have type I growth habit which is easier to handle in mechanized agricultural systems and lends itself to an once-over mechanical harvest. Plant characteristics of bush growth habit that are important for processing or fresh market beans include lodging resistance, plant height, number and length of branches, pod placement in the canopy, and length of the peduncles supporting the pods (Silbernagel, 1986). Present varieties facilitate mechanization because they have concentrated flowering and pod set, an upright growth habit with pod set midway or high on the plant, reduced foliage, strong root attachment to the soil, and resistance to some diseases (Rubatzky and Yamaguchi, 1997). Bush beans mature within a concentrated time period, approximately 50 to 60 days after seeding (Peirce, 1989).

3.1.1.2 Indeterminate Habits. Three indeterminate growth habits are distinguished (Singh, Singh, 1999; Debouck, 1991; Hidalgo, 1991; Voysest and Dessert, 1991; Schoonhoven and Pastor-Corrales, Graham and Ranalli, 1997): (i) growth habit II (indeterminate upright bush, with an erect stem and branches, and often without a guide); (ii) growth habit III (indeterminate prostrate, with well-developed branching, and low or nonexistent climbing ability); and (iii) growth habit IV (indeterminate, with long guide and high climbing ability). Most of the original edible podded beans were climbing type IV vines (Fernandez et al., Myers and Baggett, 1999) that are known as pole beans today. Pole beans occupy very little of the contemporary commercial acreage. They are more popular in China, home gardens in Europe, winter sowings in relatively warmer regions or greenhouses, and near cosmopolitan

cities in Latin America and other developing countries (Singh, 1999). Stem length in climbing types can be as long as 3 m with more than 25 flowering nodes. These forms lodge severely and thus are generally supported on poles or a trellis. Pole beans can be harvested over a longer period compared to bush types; consequently, yields usually are higher. Besides a greater yield potential, pole bean production advantages include better adaptation to high-rainfall conditions, with reduced humidity within the foliage canopy and a lower incidence of disease. Additionally, because pods are less likely to contact the soil, they are clean and grow straight (Rubatzky and Yamaguchi, 1997).

Gene action in each of these growth habits is far from being well understood, moreover, in several cases, it appears to be altered by the environment (Myers and Baggett, 1999). Type I growth habit is inherited and controlled by a single recessive gene (Norton, Debouck, 1991; Kretchmer et al., 1979). Gene action is controlled by light quality (Kretchmer et al., 1977, 1979) and also apparently by daylength (Allard and Zaumeyer, Debouck, 1991). Several genes controlling internode length have been identified (Davis and Frazier, 1966; Detongnon and Baggett, 1989); their effects seem to be additive. Lodging resistance appears to be related in part to internode length and has been described as controlled by a single dominant gene (Bliss, 1971) or three recessive genes (Frazier et al., 1958). Other factors including root structure and stem thickness affect lodging, and suggest that lodging resistance and plant architecture should show quantitative inheritance (Kelly and Adams, 1987; Acquaah et al., 1991; Brothers and Kelly, 1993).

3.1.2 Varietal Grouping by Pod and Seed Characteristics

Pod type - perhaps the most important aspect of snap bean varieties - is the character most commonly used to classify snap beans (Myers and Baggett, 1999). UPOV (1995) comprises shape of cross-section, ground colour and stringiness as pod characteristics in varietal grouping. Nevertheless, taking into account the large diversity of pod traits within the species, which strongly determines acceptability, traits of importance include also sieve size, length, width, straightness, fibre content, stringiness, smoothness, rate of seed development, holding ability, etc. (Silbernagel, 1986). Names, such as “Green Bean”, “Wax Bean”, or “Romano” (also known as “Italian” or “Flat Podded Bean”), and “Round Podded Bean” describe some of subgroups or market classes (Myers and Baggett, 1999).

Pod shape is affected by length, cross section thickness and shape, and length of the spur and pedicel (Myers and Baggett, 1999). Lengths range from 8 to 20 cm or more, with pods of processing types being about 10-16 cm in length (Rubatzky and Yamaguchi, 1997). Widths range from less than 1 to several cm. According to cross sectional shapes of UPOV (1995), snap beans are grouped as elliptic to ovate, heart-shaped, circular and eight-shaped. Most fresh market beans are oval because this trait is associated with greater durability for shipping, and gives the straightness needed for an attractive appearance (Myers and Baggett, 1999). Processors prefer a round pod because round pods are fresher for a longer time and because there is a close relationship between sieve size, quality, and maturity when the round pods are sorted in a sieve grader. Because pod cross sectional shape is a function of pod wall

thickness and timing of development, it shows quantitative inheritance, although in a study by Chung et al. (1991), an additive action with some degree of dominance was found. According to straightness, pods may be inherently straight, curved or even "fish hooked". Straightness is important in processing beans. It is affected by plant habit, and upright bush beans and pole beans tend to have straighter pods (Myers and Baggett, 1999). Depending on variety, pod ends may have a pointed or blunt tip. The spur or remnant of the style can vary in length. Processors prefer short and straight spurs because they are easier to remove. Pod sieve size is probably the single most important factor in sorting processed beans into uniform sizes (Myers and Baggett, 1999). Sieve size categories range from one to seven and over according mainly to pod cross section thickness. Full-sieve beans generally have 50% 1- to 4-sieve size at maturity. Pod smoothness varies between high smoothness and bumpiness, and is related to pod wall fibre, rate of seed development, and also seed size and shape. Pod surface may be brilliant (shiny), opaque, or intermediate. Most snap beans are glabrous; a few exhibit some pubescence. Pod texture may be fleshy, slender, or firm.

According to colour, the following grouping of snap bean is recognized: green, yellow (wax), purple, and multi-coloured (UPOV, 1995; Myers and Baggett, 1999). Nearly all beans for fresh market and processing have pods with some shade of green. Pod colour shows genetic variation for both intensity and hue (Myers and Baggett, 1999). Colours range from light- to dark-green with hues ranging from yellow-green to a blue-green colour that is characteristic of "Blue Lake" types. Wax bean colour is controlled by a single recessive gene, but may be affected by a second gene and perhaps other modifiers (Currence, 1931). There are few purple- or red-podded varieties –either solid coloured or striped– and not used commercially (Myers and Baggett, 1999).

The amount of pod fibre and rate of development also varies. Early varieties of snap bean, then termed "String Bean", had enough fibrous strands in each suture of the pod, which had to be removed manually before cooking. The stringless character was discovered in 1870 by C. N. Keeney and has since been widely incorporated into improved snap bean varieties (Zaumeyer, Myers and Baggett, 1999). Nearly all bush beans today are stringless, while some heirloom pole beans are stringed. Stringless-type varieties also contain less wall fibre. (Nevertheless, within the United States, "String Bean" tends to remain as a genetic term to identify snap beans. The word "string" was used because of the strong string-like fibres at dorsal and ventral sutures of the pod. As seed fully matured, the pod would split open.) Within snap beans, fibre content appears to be quantitatively inherited, with reported values from 0.02% to about 3.0% of pod fresh weight for pods with acceptable quality (Silbernagel and Drake, 1978). The lowest percentages are found in the "Blue Lake" beans, with fresh market and over-mature beans exhibiting the highest percentages. Fibre content also increases with sieve size and maturity, with some varieties increasing by 20% in mature six-sieve (Silbernagel and Drake, 1978). Different modes of inheritance are reported for fibrous and stringed beans (Leaky, 1988). A single dominant gene preventing string formation (Prakken, 1934; Drijfhout, Myers and Baggett, 1999), a temperature-sensitive dominant gene (Drijfhout, Myers and Baggett, 1999), and also modifiers or other genes affecting string formation were described.

One of the major determinants of pod quality is seed size or seediness. A seed index based on the product of seed weight by length has been developed to gauge quality (Silbernagel and Drake, 1978). Thus, selection for slow seed development will prolong pod quality. Seed number is another variety characteristic; most varieties contain three to five seeds; dry or common bean types tend to have several more (Rubatzky and Yamaguchi, 1997). Seed shapes are round, round to elliptic, elliptic, and kidney-shaped, while seed coat colours can occur in numerous colours and combinations (black, white, green or greenish, grey, yellow, buff coloured, brown, red, violet) (UPOV, 1995). Varieties produced for snap bean processing usually have white or light-coloured seeds. However, white-seeded varieties do lack tannins, lignins and anthocyanins (Agbo et al., Myers and Baggett, 1999), compounds involved in resistance to mechanical stress and pathogens. Seed coat colour affects water uptake as well (Wyatt, 1977). Other important features of mature seed are thickness and adherence of the testa and cotyledon resistance to cracking; resistance to cracking is genetically linked with seed coat colour (Dickson and Petzoldt, 1988).

Subsequently, genetic improvements in pod quality have included development of fibreless pod walls, further improving pod shape, slow seed development, tenderness, and white seed coat, which ensures a clear liquid component of canned snap beans. Interestingly, consumers have strong preferences for snap bean pod shape and colour, and seed colour. Each of these characteristics may have a determining influence on the acceptability of a specific variety. Pod-type acceptability is also very strict for processing beans, because even a slightly different processing texture or colour may limit acceptability. Seed colour is of little significance for fresh use pods, but white-seeded varieties are preferred for canning and dark-seeded varieties for freezing. Processed whole pods are a high-value product. Other factors, such as growth habit, stress tolerance, organoleptic factors, or yield, interact with the pod-type characteristics to determine overall acceptability in a given production zone or consumption area.

3.2 Varietal Grouping by Utilization or Mode of Consumption

Generally, snap beans may be grouped according to use on the basis of the stage of plant maturity when they are consumed, and on the basis of market requirements. The first grouping classifies snap beans into (Voysest and Dessert, 1991; Rubatzky and Yamaguchi, 1997): (i) horticultural beans, grown for and consumed as fresh or processed, preferably fibreless, immature pods; and (ii) green shell or fresh grain beans, specifically grown for and consumed as fresh, full-sized seeds. The second grouping by Silbernagel et al. (1991) classifies snap beans into five major classifications and uses: (i) home garden types; (ii) fresh market types; (iii) shipper types; (iv) processing types; and (v) freezing types.

Snap beans, consumed as fresh or processed immature pods, are a particularly important class in developed countries, such as those of Europe and North America. Other names, more or less synonymous, given to this group include “Garden”, “Green”, or “Haricot” beans, and in some cases “French” beans (Voysest and Dessert, 1991). In the older literature, “Kidney” bean may refer to an edible podded

bean (Hedrick, Zaumeyer, Myers and Baggett, 1999). The term “String” bean refers to older snap bean varieties that had fibre in the pod suture.

Green shell beans are of little importance throughout the world (Peirce, 1988; Voysest and Dessert, 1991). Shelled bean varieties are harvested when they are close to physiological maturity but still succulent and consumed in the green shell state. The seeds are separated from the pods; the latter discarded because they are fibrous and not succulent. Shelled snap beans have the characteristic of remaining firm following cooking, much like that of most cooked common dry beans and unlike the sluffing and softening of cooked snap beans (Rubatzky and Yamaguchi, 1997). Shell fresh beans are indeed a preferred food in many areas of Africa and South America, but their use is limited because of perishability (Voysest and Dessert, 1991). Seeds of this class are usually large and bicolored, often having large, fleshy red-striped pods. Other names for this group are “Horticultural” or “Borlotto” beans. “Flageolet”, “Canellini” and “Cranberry” beans also fall into this class, but they have small, light-coloured seeds.

Today, with the popularization of the processing industry, much of the pod-type classification is caused by the sophisticated requirements of the food processing industry. Specific varietal characteristics are required, depending on eventual utilization: whether for processing, whole or sliced; for canning or freezing; or for shipping of local market or garden bean types. Silbernagel et al. (1991) describes some major classifications and uses of snap beans in the United States and Europe, as follows:

3.2.1 Home Garden Types

Hundreds of varieties, some of which are very old (heirloom cvs.), are used by home gardeners, primarily for family use. These cover a wide range of pod sizes, shapes, colours, and flavours, and may range from determinate bush to indeterminate, extreme climbing vines.

3.2.2 Fresh Market Types

These types are produced commercially and are close to market outlets. They include varieties of a wide range of pod and plant characteristics, i.e. from bush to vine habits, from flat to round podded, green, yellow, purple, or multi-coloured, stringy or stringless, fresh podded or fresh grain shell types.

3.2.3 Shipper Types

Suitable varieties to the shipping trade are those which appear fresh-looking after several days in transit and in market displays. Shipper types may cover a wide range of pod characteristics, provided they are tolerant to ambient temperatures, wind scaring and other marketing blemishes. They may include fresh market or home garden types, which are on the borderline of having too much pod wall fibre, and which may develop a weak string with advancing maturity. Thus, good shippers seldom can be used by the processing industry.

3.2.4 Processing – Freezing Types

The rapid increase in popularity of processed food in the past fifty years highly influenced variety development of snap bean. Initially, all beans for processing were hand-picked home and/or fresh market types, with light to medium green colour, which were unattractive as a frozen product. With the introduction of "Tendercrop" in 1958, the frozen food processors had a product with a uniform and very appealing bright green colour. Later breeding and selection for high plant-pod quality standards (stringlessness, low pod wall fibre content, slow-seeding, suitability to mechanical harvesting, etc.) led to the development of different rapidly improved varietal groups for processing industry, which may be canning, freezing or dual-purpose types that can be either canned or frozen. Overall requirements for frozen beans are very similar to canning beans, and in fact many varieties are dual-purpose types.

Some primary requirements that are absolutely necessary in order to meet the marketing objectives of processing industry, which for the most part are predetermined before planting, are as follows:

1. Precision in scheduling plant operations. A steady flow of constant-quality and quantity raw product has to be available throughout the season. Therefore, uniformity of emergence, development and pod maturity is absolutely necessary.
2. Suitability to machine harvesting, which requires a concentrated maturity, where majority of the pods are ready at the same time for an once-over destructive harvest, strong upright plant architecture, and pods in the mid to upper part.
3. Acceptable processed product. Because market competition is so keen, the quality standards of buyers are quite high. Major pod-seed characteristics determining final product quality are as follows: smoothness, pod length of 12-15 cm, straightness, stringlessness, slow-seeding, slow fibre development, high holding ability when harvesting schedules are interrupted, small seed cavity, seed weight kept under 10% of total pod weight, and uniform internal and external colour.

4 Genetic Resources

Diversity among *Phaseolus* species in relation to common bean is organized into primary, secondary, tertiary, and quaternary gene pools (Debouck and Smartt, 1995). Each domesticated species constitutes a primary gene pool with its wild ancestral form. Secondary and tertiary gene pools may exist for all domesticated species, depending on the phylogenetic events that lead to the biological species (Debouck, 1999). The primary gene pool of common bean comprises both varieties and wild populations (Singh, 2001). Hybrids between the wild and cultivated beans are fertile and have no major barriers (Motto et al., 1978; Singh et al., 1995). The secondary gene pool of common bean comprises *P. coccineus*, *P. costaricensis* Freytag and Debouck, and *P. polyanthus* (Singh, 2001). Hybrid progenies between crosses of common bean and any of the three species forming the secondary gene pool may be partially sterile, preventing the recovery of desired stable common bean phenotypes (Wall, 1970; Manshardt and Bassett, 1984). The tertiary gene pool of common bean comprises *P. acutifolius* and *P. parvifolius* Freytag (Singh, 2001). Hybrid progenies between crosses of common bean and any of the two species forming the tertiary gene pool require embryo rescue (Singh, 2001). The quaternary gene pool of

common bean comprises *P. filiformis*, *P. angustissimus*, and *P. lunatus* (Singh, 2001). Crosses of common bean with these three species have been attempted without producing fertile viable hybrid progenies (Singh, 2001).

Genetic diversity in common bean is organized in large-seeded Andean, and small-and medium-seeded Middle American gene pools (Evans, 1980). Further evidence for the existence of the two gene pools was provided by the relationship of seed size (small versus large) with (i) the Dl genes (Dl-1 versus Dl-2) and the F_1 hybrid incompatibility, (ii) phaseolin seed proteins, (iii) allozymes, (iv) morphological traits, and (v) DNA markers (Singh, 2001). Singh (1989) described in detail the patterns of variation in common bean varieties. Singh et al. (1991a) further divided the Andean and Middle American cultivated gene pools into six races: three Andean (all large-seeded); three Middle American (medium- or small-seeded), each with distinguishing characteristics, ecological adaptation and agronomic traits. Although some contemporary snap bean varieties are originated in the Andean gene pool, they are actually intermediate between the two gene pools as shown by molecular marker analysis (Skroch and Nienhuis, 1995). For decades, breeders have crossed extensively the Mesoamerican and Andean germplasm primarily in Europe, in the USA, or both (Myers and Baggett, 1999). Improvements in snap bean include a change from climbing to bush growth habit, concentration of pod set, small seed and pod size, and other traits (Silbernagel, 1986; Silbernagel et al., 1991; Myers and Baggett, 1999; Myers, 2000;), as well as resistance to diseases and to some abiotic stresses (Silbernagel, 1987; Silbernagel et al., 1991; McMillan et al., Singh, 2001). At present, the information available on genetic relatedness and ancestry in snap beans is scattered (Myers and Baggett, 1999).

Since the 1950s a number of agencies and organizations, especially the Food and Agriculture Organization (FAO) of the United Nations, have promoted and supported exchanges of germplasm and related information and technology, involving all countries of the world (Esquinas-Alcázar, 1993). The non-governmental International Agricultural Research Centres established under the aegis of the Consultative Group of International Agriculture Research in the 1970s, have also promoted and facilitated international technical co-operation for the genetic resources of crops under their mandate. The historical background of collection of *Phaseolus* bean germplasm dates back to 1970 when, with the creation of the Centro International de Agricultural Tropical (CIAT), a Food Legumes Production Systems Program was initiated. Aside from the obvious task of gathering as much as possible of the existing variability in *Phaseolus* beans, a major challenge is to quantify the germplasm variability, especially for the common bean. Another challenge is to make effective use of the related species (secondary and tertiary gene pools), as a means not only to improve the common bean but also to use those species as crops in environments where they are better adapted than *P. vulgaris* (Hidalgo, 1991). A better understanding of real variability and phylogenetic relationships is a powerful tool that bean researchers (e.g. breeders) can exploit in using the available germplasm base more efficiently. Since the establishment of the gene bank in 1973 until 1991, CIAT distributed 47300 samples of *Phaseolus* beans to 83 countries on five continents (Hidalgo, 1991). Also, over 362 CIAT or CIAT-derived varieties have been released in more than 39 countries (Broughton et al.,

2003). CIAT has a mandate to conserve over 30000 accessions of domesticated and wild common bean lines (Broughton et al., 2003), as well as newer collections made in collaboration with the International Board for Plant Genetic Resources (IBPGR; now IPGRI) (Graham and Ranalli, 1997). The IPGRI databases contain passport data of over 30000 accessions representing the *Phaseolus* collections maintained in European genebanks (http://www.genbank.at/phaseolus). The accessions are maintained under two types of storage: short- to medium-term working stocks stored at +5ºC; and long-term stocks sealed in laminated bags at 5-8% moisture content, and stored at -20ºC (Hidalgo, 1991). The germplasm is characterized and evaluated on the basis of 25 plant and six seed descriptors (Hidalgo, 1991).

5 Major Breeding Achievements

Pole and bush beans with green and yellow pods are found in Europe from an early date, suggesting that much of the variation found in the early American varieties was already present in Europe (Myers and Baggett, 1999). Thus, the edible podded bean reintroduced into Americas from Europe (Gepts et al., 1988). The first varieties in Europe and the USA were selected from variation generated by mutations and chance outcrosses in older varieties. These were often multiple purpose beans, being consumed as immature pods, mature shell beans, and as dry beans (Myers and Baggett, 1999).

After the release of the first round-podded variety in 1865 (Silbernagel, 1986) and the first stringless variety in 1887 (Atkin, 1972), the most significant breakthrough in snap bean improvement came with Zaumeyes in 1958 (Silbernagel, 1986), who released the cv. Tendercrop. Tendercrop was the first slender-podded snap bean, with a strong upright bush, suitable for new industry standards, that is still used today as ancestor for many varieties of the frozen bean processing industry (Silbernagel, 1986). In 1970, 46% of the green-podded bush types had Tendercrop germplasm in their ancestry (Zaumeyer, 1972). Another factor that influenced variety development in the past three decades was the rapid increase in popularity of frozen foods. With the introduction of Tendercrop, the frozen food processors had a product with a uniform and very appealing bright-green colour. In the early 1950s, M. Parker released the cv. Gallatin 50, an off-white seeded variety from Tendercrop, which was the leading canning variety for about 10 years, until replaced by the white seeded cv. Early Gallatin (Silbernagel, 1986). Prior to the late 1950s beans were picked by hand two to six times during the season. In the mid-1960s, Pierce's cv. Harvester was released. It was an early variety combining fresh-market characters with adaptation to once-over destructive harvest (Silbernagel, 1986).

A group of varieties called Blue Lake beans (Myers and Baggett, 1999) have been synonymous with dark green pods, distinctive mild flavour and texture, and the ability to preserve their appearance after processing. The "Blue Lake" name apparently derives from the Blue Lake area near California. The first cultivated snap bean varieties in this region were Scotia, introduced from Germany in 1896 and White Greaseback, originated in America (Hedrick, Myers and Baggett, 1999). W.A. Frazier was one of the first breeders who transferred the pod quality characteristics from pole-type Blue Lake varieties to bush types adapted to mechanical harvesting (Frazier, et al., 1958). The first bush Blue Lake types were introduced in

1965 (Baggett, 1995), but they were not a success because of a poor growth habit, while the first successful release was in 1970 (Frazier, et al., Myers and Baggett, 1999). A review of major breeding achievements would not be complete without mentioning Anderson's varieties, more than 40 new varieties that dominated the industry for many years (Silbernagel, 1986). During the past half-century breeders used important original sources of resistance. For instance, the breeders efforts for transferring resistance to beet curly top virus (BCTV), started in 1930s and 1940s, and resulted in the release in 1943 of resistant snap bean varieties by Pioneer (Larsen and Miklas, 2003). In 1970, the Bean Improvement Cooperative at USA presented Anderson, Frazier, Parker, Pierce, and Zaumeyer with the Meritorious Service Award, in recognition of their outstanding contributions to the U.S. snap bean industry for about 40 years (Silbernagel, 1986).

6 Current Goals of Breeding

An ever lasting breeding goal for snap bean varieties consists the confrontation of environmental effects which include abiotic factors and biotic stresses. On one hand, stresses reduce crop yield and quality rendering sometimes an entire field unmarketable. On the other, all the commercially acceptable snap bean varieties retain some level of susceptibility (Jung et al., 2003). Silbernagel (1986) stated that a snap bean variety can never be a perfect one, because different end uses have different requirements, and the horizons are broadened and the goals are set higher as the current objectives for any particular end use are approached. Moreover bean breeders need to determine not only what is needed, but also, what else will be needed in 10-15 years when the new variety will be introduced. Silbernagel (1986) suggested the collaboration between commercial breeders who must integrate the problems of growers, shippers and/or processors focusing on managerial economic considerations, and public breeders who must direct their research to areas of long-range needs. The following analysis of current goals will touch on the manipulation of diversity of stresses, considering some of the common objectives sought by all breeders.

6.1 Abiotic Stress Tolerance

Abiotic stress management is one of the most important challenges facing agriculture. Major abiotic stresses limiting snap bean crop yield are: high and low temperature, drought, salinity, soil nutrient deficiency, nitrogen fixation, and air pollution. Stressful environments are often characterized by the simultaneous occurrence of more than one stress. The tolerance of plants to stress has been widely shown to vary with physiological growth stage, developmental phase, and size of plants.

6.1.1 Cold Tolerance

Tolerance to freezing temperature varies with multiple factors, and it is difficult to determine how much freezing damage is the result of temperature alone. A crop species may have tolerance varying with different growth stage, duration of freezing

temperatures, soil moisture, acclimation/declamation cycles, and other associated factors (Meyer and Badaruddin, 2001).

Beans are generally susceptible to low temperatures injury at all stages of growth (Silbernagel, 1986). Temperatures of 10°C or below during imbibition and germination may result in permanent injury and vigour reduction, while prolonged temperatures at or below 15° to 16°C can result in stunted plants with no crop (Dickson and Petzoldt, 1987). Cold stress at reproduction phase, i.e. at flowering, is largely associated with either the abnormal development of flowers or the failure of pods to set seed (Blum, 1988). Generally, most snap bean varieties need a period each day above 16°C for pollen tubes to grow sufficiently for fertilization and for subsequent pod development. However, studies showed that low night temperature of 8°C does not prevent pod set.

Generally, delayed planting of beans may cause greater economic losses than does frost through the reduction of yield and quality of seed (Blaylock, 1995). The ability of plants to survive under cold conditions depends on their capacity to increase the activity of their antioxidant enzymes. Cold tolerance is a complex trait whose inheritance is under genetic control (Tokuhisa and Browse, 1999; Thomashow, 2001). Most genetic studies have been performed at the early stages of plant development. A factor contributing to the complexity of cold tolerance inheritance is the strong interaction between genotypes and environments.

It has generally been assumed that the inheritance of cold tolerance is polygenic (Fowler et al., 1999). Additive effects have been reported as the most important gene effects involved in cold tolerance of common bean (Otubo et al., 1996). Although several selection procedures for cold tolerance have been developed (Austin and MacLean, 1972; Hardwick and Andrews, 1980), they are plant-destructive or not adapted to selection among large numbers of plants. Dickson and Boettger (1984a, b) found that germination of seeds at 5°C for 5 days, followed by growth of plants at 16°C, was correlated well with field performance under cool conditions. Farlow et al. (1979) observed that low day temperatures reduced seed number per pod due to slow pollen growth. Narrow sense heritabilities were 28%, 56%, 45%, and 74%, for imbibition at 5°C and 16°C, seedling vigour, plant vigour, and days to bloom, respectively, in a cross of snap bean varieties (Dickson and Petzoldt, 1987). The heritability value at blooming showed that the tendency of a line to set pods at 16°C indicating the ability of pod setting at low temperatures was recessive and quantitative. Intrapopulation recurrent selection or even mass selection are therefore recommended for improving cold tolerance since additive effects are the most important gene effects.

As the final expression of the genetic potential conditioned by environmental factors, yield under cold conditions is the best trait to evaluate cold tolerance. Two species, *P. filiformis* and *P. angustissimus*, are able to survive subzero temperatures at the seedling stage. In a breeding program at Canada (Broughton et al., 2003), interspecific crosses of the above species with *P. vulgaris* were made for transmitting the ability to withstand the subzero temperatures to the hybrids, and several lines with improved ability to germinate at low temperature were identified.

6.1.2 Heat Tolerance

Heat stress particularly affects the development of reproductive organs (Hall, 1992). For snap bean research workers it is widely accepted that high temperatures disrupt the pollination-fertilization cycle (Bouwkamp and Summers, 1982). The optimum pollen germination temperature has been shown to be 15^{o}C with germination inhibition in sensitive varieties at 30^{o}C (Admad, Bouwkamp and Summers, 1982). A study on flowers that had abscised at high temperatures showed that fertilization had not taken place. This led to the conclusion that blossom abscission may be due to the inability of pollen grains to germinate at high temperatures. High air temperature affected the endoplasmic reticulum structure and blocked its function in the tapetum, and then induced earlier than usual degeneration of tapetum (Suzuki et al., 2001). Pollen sterility was associated with tapetal degeneration, when pollen sterility was induced by high air temperature, 8 to 11 days before flowering in the snap bean (Suzuki et al., Suzuki et al., 2001). In particular, it was observed that, when the average air temperature during that period exceeded 28^{o}C, more that 80% of the pollen produced was sterile. A high negative coefficient of correlation between temperature (15^{o}C-35^{o}C) and the percentage of pods set was estimated (Iwani, Bouwkamp and Summers, 1982).

Pod yield of snap bean is severely depressed under a high temperature condition. It is determined by the number of pods, which is a product of the number of flowers and pod-set-ratio. Since pod-set-ratio is strongly affected by pollen fertility under high temperature condition, pod yield deterioration in the summer cropping might be due to the decrease of pollen fertility. Often a high temperature coincides with a high solar radiation and causes excessive transpiration. This excessive transpiration leads to temporal water deficit in plants in the daytime, even when soil moisture content is adequate and plants can take up a sufficient amount of water in the nighttime. Decline in water potential of vegetative and reproductive organs in snap bean plants was considerably larger under high temperature than that under optimal temperature conditions (Tsukaguchi et al., 2003). These findings suggest that heat-tolerant snap bean varieties maintain a good water status in the daytime. Water-uptake ability is determined by the product of root surface area and root activity. Better growth of a heat-tolerant snap bean variety under high temperature conditions was due to its higher photosynthetic rate in the daytime. The higher stomatal conductance of the resistant variety was attributed to higher water uptake rate. The cooler leaf of heat-tolerant varieties compared with that of heat-sensitive varieties suggests that, leaf temperature could be a useful criterion for the selection of heat-tolerant snap bean varieties (Tsukaguchi et al., 2005). The mode of inheritance to stress caused by high temperature was due to a single dominant gene and two genes with epistatic action (Bouwkamp and Summers, 1982). Sources of tolerance to high temperatures during bloom have been reported (Silbernagel, 1986). It is possible to recover heat-tolerant single-plant selections from advanced generation hybrid populations derived from heat-tolerant lines (Silbernagel, 1986). Nevertheless, the combination of resistance from both parents did not appear to result in increased resistance (Bouwkamp and Summers, 1982). Rainey and Griffiths (2005) studying the mode of inheritance of heat tolerance during the reproductive development in snap bean supposed that it

may be influenced by major genes. They indicated a six-parameter model that includes non allelic interaction terms, perhaps the result of the abscission gene interacting with other genes for pod number. A simple additive/dominance model accounted for genetic variance for seeds per pod.

6.1.3 Drought Tolerance

Beans require between 200 and 400 mm of rainfall as comparable residual soil moisture during growth and development, with the well watered area reaching globally up to only 7% (Broughton et al., 2003). For snap beans soil moisture of 250-450 mm is usually sufficient (Rubatzky and Yamaguchi, 1997). Singh (1995) reported that water stress during flowering and grain filling reduced seed yield and seed weight, and accelerated maturity of bean. Reductions in yield during flowering are the result of both fewer pods and seeds per pod. Robins and Domingo (1956) found that water stress during the vegetative stage delayed flowering, while water stress during the reproductive and grain-filling stages hastened plant development. Also, water stress during the vegetative stage retarded root development, as well as vegetative growth. Total number of pods and pod fresh weight of bush bean were significantly reduced by water stress occurring at preflowering, flowering or post flowering stages (Dubetz and Mahalle, 1969). According to Rubatzky and Yamaguchi (1997), moisture stress also affects pod colour, fibre and firmness.

A potential source of drought stress-tolerant traits for *P. vulgaris* through interspecific hybrids is *P. acutifolius* (Haghigi and Ascher, Lazcano-Ferrat and Louatt, 1999). *P. acutifolius* possesses both morphological and physiological characteristics that enable it to complete well its life cycle and yield under hot arid conditions. However, progress in the development of tolerant lines is slow due to the lack of simple traits associated with drought tolerance. Therefore, it is important to identify the characteristic traits associated with pod setting, the number of pods reaching maturity, and the seed yield with the purpose to use as a marker to screen snap bean germplasm with drought tolerance (Omae et al., 2005). Trehalose plays a role in drought tolerance of rhizobia/legume symbioses, particularly in common beans. Nodulated plants that accumulate only small amounts of trehalose are poorly drought-tolerant, whereas those that accumulate higher concentrations are more resistant to drought stress (Farlas-Rodriquez et al., 1998). A program for studying the involvement of trehalose in drought tolerance in different bean accessions of CIAT germplasm was applied in Mexico (Broughton et al., 2003). Moreover, researchers focus on the functional characterization of genes involved in drought tolerance, especially the so-called *LEA* genes (Ingram and Bartels, 1996) for identification of molecular markers associated with drought tolerance (Broughton et al., 2003).

6.1.4 Salinity Tolerance

The common bean is a salt sensitive species (Mass and Hoffman, 1977) that is primarily grown in semiarid tropical environments and also in irrigated soils of these regions. About 20 to 30% of the bean production area in the Middle East and 5 to 10% in Latin America are affected by soil salinity (CIAT, 1992). These areas are

subjected to high salt concentrations in the topsoil, because of capillary rise and evaporation of soil water during the dry season or from salinity of irrigation water. Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Greenway and Munns, 1980). Moreno et al. (2000) studied the genotypic variability in bean varieties for resistance to salinity (sodium chloride) at the seedling stage in pots. Highly significant differences were found among genotypes and among treatments for plant height, seedling fresh and dry weight, and root length. Some of the tolerant to salinity genotypes were selected. Higher root growth and mineral acquisition in roots in the salinity stress test were related to the mechanism of resistance at the seedling stage. Wild *Phaseolus* species and in particular *P. filiformis* represent a genetic resource for improvement of salinity tolerance in common bean (Bayuelo-Jiménez et al., 2002a, b).

6.1.5 Nitrogen Fixation

One of the driving forces behind agricultural sustainability is effective management of nitrogen in the environment. The primary source (80%) of biological fixed nitrogen is through the soil bacteria *Rhizobium*-legume symbiosis (Vance, 1997). Legumes provide 25-35% of the worldwide protein intake. The micro-symbionts of *P. vulgaris* constitute a heterogeneous group of bacteria. At least five different species belonging to the genera *Rhizobium* and *Sinorhizobium* have been identified from bean nodules. The original micro-symbiont of *P. vulgaris* is *R. etli* (Segovia et al., Broughton et al., 2003). The snap bean production system is nitrogen deficiency rare, because the benefit of applying nitrogen is realized by green-pod production. The response of bean to nitrogen fertilization under field conditions indicates different *Rhizobia*-variety relationships or symbiotic nitrogen fixation and seed yield through plant breeding (Westermann and Kolar, 1978).

6.1.6 Soil Nutrients

Soil problems due to toxicities and/or nutritional deficiencies limit productivity. Beans are frequently produced on acid soils that are low in available phosphorus and/or with high phosphorus fixing capacities. Such soils are often high in aluminium and beans are affected by aluminium toxicity. Beans are, also, affected by manganese toxicity and low availability of nitrogen. In bean varieties the distribution of dry matter and nitrogen content is related to the end-use product of each of them (Ninou and Papakosta, 2006). Cichy et al. (2005) determined that a single dominant gene controls the high seed zinc concentration in a bean cross. Zinc efficient genotypes of bean loaded more zinc into seeds than zinc inefficient genotypes in field experiments (Moraghan and Grafron, 1999). Although little is known of the significance of these micronutrient balances in the bean production system, preliminary observations indicate that it is also about the same as for potassium (Broughton et al., 2003). Bean varieties vary in their sensitivity to nutritional disorders; but only limited data exists to indicate high tolerance (Hagedorn and Inglis, 1986).

6.1.7 Air Pollution Tolerance

Under some areas snap bean production was limiting owing to air pollutants (Silbernagel, 1986). The principal pollutants are sulphur dioxide and ozone (Myers and Baggett, 1999). Ozone enters the plant through open leaf stomata and then rapidly decomposes to form reactive oxygen intermediates within the cell wall. Ascorbic acid in the leaf apoplast has the potential to limit ozone injury by participating in the reaction that detoxifies ozone and reactive oxygen intermediates, and thus prevents plasma membrane damage. Elevated extra cellular abscorbic acid was associated with ozone tolerance in a series of snap bean genotypes (Burkey and Eason, 2002). Crossing improved well-adapted varieties as parents and screening of segregating populations under polluted conditions would greatly increase the probability of finding tolerant selections and incorporate the resistance (Silbernagel, 1986). Resistance to ozone damage is quantitatively inherited, and reported to be controlled by recessive genes with high heritability (Knudson-Butler et al., Myers and Baggett, 1999), or as an additive trait of moderately high heritability with possible epistasis (Myers and Baggett, 1999). Bean varieties vary greatly in reaction to ozone or air pollution tolerance according to the time and place field evaluations are made.

In the air pollution the sunscald and wind injury were added. Rarely sunscsald occurs (Hagedorn and Inglis, 1986), almost everywhere beans are grown, and rarely causes a significant economic loss. Wind injury occurs on beans (Hagedorn and Inglis, 1986) and causes economic losses when pod damage occurs on processing varieties. Tall, large-leaved bean varieties with a substantial “Blue Lake” genetic background and determinate processing varieties show more pod injury.

Studying abiotic stress factors, breeder’s eye notifies common points: (i) wild *Phaseolus* species and in some cases *P. vulgaris* varieties represent a genetic resource available to selection procedures, (ii) the mode of inheritance of tolerance appears to be quantitative and in many cases reported to be controlled by recessive genes with high heritability, and (iii) evaluations and tests for tolerance are usually applied at seedling stage, thus genotypes seldom reach field experiments to confront genotype per environment interaction. These points represent a synopsis of breeding challenges for future breeder task in order to follow Silbernagel (1986) statement that snap bean breeder needs to maintain genetic factors for maximum productivity and quality under good, stressful and disease cultivation conditions.

6.2 Biotic Stress

Systematic evaluation of wild common beans, as well as wild and domesticated germplasm of alien species for resistance to pest, diseases and virus has been limited (Broughton et al., 2003). Moreover, breeding for genetic resistance to many pests and diseases almost inevitably reduces the emphasis that can be given to the improvement of yield potential. The most effective long-term strategy is probably to combine the use of agrochemicals and genetic resistance in order to optimize the use and extend the effective life of both (Evans, 1993).

6.2.1 Response to Density

Narrow row spacings and high-density plantings have increased the seed yield of some crops. Atkins (Grafton et al., 1988) obtained yield increase of snap bean with narrow row spacings. Mack and Hatch (1968) obtained the highest yields of snap bean, when plants were spaced in an equidistant arrangement (0.13 m spacings). Clothers and Westermann (1976) reported high seed yields of determinate snap bean varieties, when plants were grown in a nearly equidistant arrangement. However, yields were not increased, when the varieties were indeterminate type, suggesting that indeterminate types have more yield component compensation than determinate types. Westermann and Clothers (1977) found that seed yield of the indeterminate varieties remained constant over a wide range of plant populations due to significant changes in the yield components. Therefore indeterminate genotypes would not respond as favourably to higher planting densities as would determinate genotypes. Spacing studies by Mack and Stang (Silbernagel, 1986) showed that maximum production was obtained when each plant had an average of 36 in^2 of space in a nearly equidistant arrangement. Commercial experience has shown that population of 160000-170000 plants/acre in various spacing arrangements can increase twice the production (Silbernagel, 1986). Current varieties are not ideally suited to this production practice and the characteristics that presumably would contribute to even more efficient and higher production levels are being identified (Silbernagel, 1986). The best way to affect future gains to yielding ability may be to make further improvements in tolerance to high plant densities, in combination with improvements in potential yield per plant under low-stress environments (Duvick, 1997).

6.2.2 Response to Insects

Beans are host to a wide range of insect pests including Aphidae, Hemiptera, and Coleoptera. Insect damage is caused by direct feeding on leaves, damage to developing pods, damage to the stem and through the transmission of virus and bean dwarf mosaic virus. Methods used to control insects in beans include the use of pesticides, cultural practices and biological control. Pesticides only poorly control aphids, thrips and whiteflies, due to the rapid development of insecticide resistance (De Barro et al., Broughton et al., 2003). Increasingly, varieties of plants resistant to insect attack are being used as a method to reduce losses caused by insect feeding and to reduce the population density of pest developing on crops (Carozzi and Koziel, 1997).

Resistant plant varieties can be used as the primary method of insect control, or as a component of an integrated pest management program (Wiseman, 1994). Insect resistant varieties have been developed to pre- and post-harvest damage by beetles (Beebe et al., 1993), by leaf-hopper species with long-term recurrent selection at CIAT in Colombia, and the derived varieties showed multiple resistance to insect attack (Bueno et al., Broughton et al., 2003).

6.2.3 Response to Diseases

The most economically significant bacterial disease of processing beans (Sherf and Macnab, 1986) is *Pseudomonas syringae* pv. *syringae* (Van Hall) bacterial brown spot. Besides, bacterial wilt, *Corynebacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Dows, common blight, *Xanthomonas campestris* pv. *phaseoli*, and halo blight, *Pseudomonas syringae* pv. *phaseolicola* (Burk) Dows (with two races) cause losses of the production. Quantitative inheritance patterns for the reaction to *P. syringae* pv. s*yringae* have been reported (Hagedorn and Rand, Hagedorn and Inglis, 1986). A gamete selection program for introgression and pyramiding of resistance to both bacterial blights with multiple-parent populations was applied in beans (Asencios-Manzanera et al., 2006). The program resulted to a number of resistant lines and in some of them with higher resistance to both bacterial blights than their parents indicating transgressive segregation.

Fungus-incited foliage diseases are: alternaria leaf spot (*Alternaria alternata*), angular leaf spot (*Isariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), ascochyta leaf and pod spot (*Ascochyta boltshauseri* Sacc. and *A. phaseolorum* Sacc.), rust (*Uromyces phaseoli*), and white mold (*Sclerotinia sclerotiorum*). High heritability estimates (0.73 straw test, 0.62 field reaction) were observed for *Sclerotinia* white mold disease in snap bean F_5-F_7 recombinant inbred lines (Miklas et al., 2001, 2003). Selective mapping of RAPD markers detected two QTLs, which were associated with canopy height and lodging traits that condition disease avoidance.

Root and stem diseases are: *Fusarium* root rot (*Fusarium solani* f.s.p. *phaseoli*), *Pythium* (*Pythium* spp.) damping-off, *Rhizoctonia* (*Rhizoctonia solani*), and root-knot nematode (*Meloidogyne*). Root disease becomes more severe when bean roots are unable to escape the pathogen due to edaphic factors. Low temperatures, drought, flooded or water logged conditions and soil compaction can hamper root growth and predispose bean plants to severe *Fusarium* root rot infection. Seed yield loss is especially severe when the disease occurs during flowering and pod fill. When the primary root dies due to infection, its function could be replaced by roots that arise from the shoot-root transition zone and generally adopt a horizontal rather than a vertical orientation. These basal roots are frequently referred to as adventitious roots, although, by definition, adventitious roots arise only from hypocofyl tissue. A root system with many horizontal roots has been termed topsoil foraging architecture with competitive advantage for phosphorous acquisition in the topsoil. Promoting lateral and adventitious roots may also contribute to plant survival in the presence of root rot organisms. Plasticity of root system response was high indicating the value of screening in the field environment. Breeding for root-rot resistance has received considerable attention, but genetic linkage seems to occur between resistance and undesirable plant characteristics. Resistance may be controlled by three to seven dominant genes (Bravo et al., 1969), few acceptable varieties are available as greater number adventitious roots can contribute to root rot resistance (Roman-Aviles et al., 2004).

6.2.4 Response to Viruses

The most commonly virus diseases (Hagedorn and Inglis, 1986) that caused considerable loss in many bean growing areas are: bean common mosaic virus (BCMV), beet curly top virus (BCTV), sometimes called *Ruga verrucosous*, bean golden mosaic virus (BGMV), and bean yellow mosaic virus (BYMV). Alien germplasm seems to be a promising source of common bean improvement for resistance to BGMV, as *P. polyanthus*, *P. costaricensis*, and *P. coccineus* that might be sources of resistance genes (Singh, 1999). Important original sources of resistance to BCTV in bean includes landraces from which released the first BCTV-resistant snap bean varieties (Larsen and Miklas, 2003). Resistance was conditioned by a single dominant allele tentatively designated *Bct* (Mackie and Esau, Larsen and Miklas, 2003). Nowadays, marker-assisted selection (MAS) for *Bct* resistance to BCTV should considerably reduce the time currently required for screening germplasm using field trials (Larsen and Miklas, 2003).

7 Breeding Methods and Techniques

7.1 The Strategics of Breeding

The strategics of breeding aims to establish the necessary framework of knowledge and techniques that will result to a snap bean variety with high yield, high-stability, and high-quality product, desired by the local and consumer communities. Thus, increasing bean yields has important repercussions on improving nutrition and health of hundreds of millions of bean consumers in the world.

7.1.1 The Methodology for Developing Lines

The purpose of most crop breeding programs is to increase and/or stabilize the harvestable yield per unit. Physiological, morphological, and yield components are often suggested as selection criteria for maximizing yield (Singh, 1991). The selection method was determined by the number of traits to be combined together and their heritability (Singh, 1992). Yield was found to be the best selection criterion in common bean (Singh, 1992). Thus, any effort to breed high-yielding bean varieties must take into account the principal characteristics of a variety' s gene pool, i.e. specific seed type (seed size, colour, form, etc.), growth habit (determinate bush, indeterminate bush, indeterminate climbing), maturity class (early, intermediate, late), and growing environment (stress vs. non-stress), and agronomic management (Singh, 1991). Variety improvement for overall performance is achieved by simultaneous accumulation of genes for yield promotion and tolerance to adverse factors (Singh, 1991).

A large body of empirical evidence has been accumulated to demonstrate that classical selection methods have been efficient in obtaining genetic improvement in cultivated crops (Moreno-Gonzales and Cubero, 1993). Data from long-term selection experiments indicate that genetic variance does not become exhausted and improvement is continuous (Dudley and Lambert, 1992). It seems that in long-term

selected populations, the depletion of genetic variance has not been critical in limiting their genetic improvement. Also, it is generally considered that yield is under mutagenic control and that yield potential progress is mostly gradual (Evans and Fisher, 1999).

From recent reviews (Silbernagel and Hannan, 1988, 1992; Debouck, 1999), it is obvious that only a small portion of genetic variability available in *Phaseolus* species has been utilized thus far for common bean improvement, since organized breeding was initiated in the late nineteenth and early twentieth century (Singh, 2001). Major breeding objectives in snap bean concern the development of varieties combining high productivity, stable yields, earliness, pest and disease resistance, tolerance to environmental stresses, and desirable agronomic-horticultural attributes (Silbernagel, 1986; Singh, 1992). The achievement of such objectives should take into account the cropping systems, the ecological conditions, and the preference of consumers in the target areas. In temperate areas of monocropping and intensive cultivation, mechanical cultivation, and industrial processing of the final product have directed efforts to breed varieties with determinate bush type, short duration, concentrated pod maturation, and uniformity in plant height, seed shape, and size.

Much of the genetic improvement of snap bean has been achieved through the selection of varieties by applying conventional breeding techniques of self-pollinated crops (Singh, 1992), such as bulk or mass, genealogical or pedigree, backcross, and their modifications (Brim, 1966), such as the single seed descent method. Conventional pedigree selection based on visual evaluation may be difficult, especially for traits with low to moderate heritability, such as seed yield (Patino and Singh, 1989). Bulk breeding methods were effective for endowing genotypes with a better yield stability (Allard, 1961; Allard and Bradshaw, 1964). However, the highly competitive variety derived from such population did not necessarily give the best seed production (Tucker and Webster, 1970; Tucker and Harding, 1974). Population improvement based on recurrent selection techniques has greater potential than the preceding methods for the introduction of quantitatively inherited traits, such as seed yield or horizontal resistance to pests and diseases (Baudoin, 1993). In some studies, recurrent selection was found to be ineffective for improving seed yield in common bean (Sullivan and Bliss, 1983), while others justify the use of recurrent selection in interracial and inter-gene-pool populations for improving seed yield efficiently (Singh et al., 1999; Beaver and Kelly, 1994; Kelly and Adams, 1987). Singh (1994) showed that gamete selection for simultaneous improvement of multiple traits, including seed yield, from early generations was superior in efficiency to other methods, including conventional pedigree, single seed descent, and mass selection. Urrea and Singh (1994) found that the F_2-derived family method of selection was superior to the single seed descent and bulk methods commonly used for advancing early generation of hybrid populations.

Developments in biometrics have suggested that the early generation trials may be used to predict the ranking of the crosses according to their likelihood to produce superior recombinant lines (Jinks and Pooni, 1976). Identification of superior crosses or populations from which high-yielding lines could be derived is of utmost importance. Many breeders used the dialell cross technique to assess the usefulness of parent and early-generation progeny performance for identifying the most

promising crosses. However, applying cross predictious methods in bean is contradictory. Hamblin and Evans (1976) reported that yield of parents and/or early generation bulks of hybrid populations was useful for identifying high-yielding populations. Quinones (1969) reported positive association between mean parential performance and the F_8 lines selected from their crosses. Singh and Urrea (1995) and Singh et al. (1990) suggested selection for seed yield in early generation of interracial and intergene pool populations to identify promising populations with desirable recombinants. From early generation yield tests (F_2-F_4), Singh and Terán (1998) identified high- and low-yielding populations that eventually produced high- and low-yielding advanced generation (F_7) lines. Visual selection for seed yield in individual plants of the F_2 and progeny rows of F_3 was ineffective (Patino and Singh, 1989), while the mean performance of parents alone was inadequate to predict yield of their crosses (Ramalho et al., 1988). High yielding, small-seeded varieties, usually possessed zero or negative general combining ability for seed yield (Nienhuis and Singh, 1988), and yield selection in crosses among them was ineffective (Singh et al., 1989b). Germplasm with medium-sized seed possessed positive general combining ability (Nienhuis and Singh, 1988). The aforementioned data led Singh (1989) to conclude that in common bean, parental yield, parental general combining ability, and early generation yield testing of hybrid populations and/or families should be considered simultaneously to breed for high seed yield. Final, Singh et al. (1990) justified these prerequisites using yield test of early generation bulks for identification of high-yielding populations that could be saved for single plant selection in subsequent generations.

The snap bean, as a vegetable, concerns plant breeders for its yield, maturity and horticultural attributes:

7.1.1.1Yield Attributes. Mechanical harvesting can only occur once in the life of the crop, whereas hand picking can take place on a daily basis. The extra-fine and fine quality of snap bean preferred in France can be obtained by daily picking of immature pods (Silbernagel, et al., 1991). Olufayo et al. (1981), and Seth and Wareing (1967) showed that picking of immature pods can stimulate compensatory increases in pod production. In *P. vulgaris* all flowers, given the opportunity, are capable of producing pods (Binnie and Clifford, 1981), but normally only the first set pods are retained, and the majority of the remaining flowers abort (Subhadrabandhu et al., 1978). Picking only twice, Olufajo et al. (1981) obtained a yield increase of 74% compared with a single pick by hand. Silbernagel et al. (1991) supported those dual-purpose varieties (producing a reasonable seed yield that could be used for consumption as dry beans after harvesting snap beans) with indeterminate bush growth habit could be developed in tropical countries. Besides, a breeding strategy for the tropics could emphasize developing varieties that are capable of responding to multiple picking by increasing pod yield. Sixty-five percent to 90% of all pods that reached full maturity were from floral buds that reached anthesis during the first two weeks of flowering (Weaver et al., 1984). Climbing varieties, being indeterminate, tend to flower over an extended period and respond to regular picking by developing new pods (Silbernagel et al., 1991). In bush snap beans varieties, yield might be improved by using more equidistant spacing arrangements (Mauk et al.,

1983), i.e. for harvesting only once, a density of up to 50 plants/m^2 is more suitable, while for the multiple-pick situation, lower density may be more suitable. Snap bean varieties with coloured seeded genotype have superior yield than white-seeded varieties suitable for the processing industry (Silbernagel et al., 1991). Much evidence indicates that resistance to root-rot-diseases and seedling vigour are physiologically linked with coloured-seed (Deakin, 1974).

For standardizing the quality grade limits in a variety, Silbernagel and Drake (Silbemagel and Drake, 1978) proposed potential yield, sieve size distribution, days/or heat units to harvest maturity, and quality. The determination of quality can be refined by using the seed index (Silbernagel and Drake, 1978). The pod production is about three times and the production of unripe seeds about twice the value of the weight of the ripe seeds (Grubben, 1977).

7.1.1.2 Earliness – Maturity Attributes. Common bean is usually considered among the earliest maturing crops. Also, farmers often express a strong preference for early maturing among bean varieties. Earliness may serve not only as a mechanism for avoiding late season stresses such as water deficits or frost, but may have economic value depending on price fluctuations or the needs of farmers for rapid sources of food or marketable products (Cerna and Beaver, 1990; White and Singh, 1991). Many bean breeding programs in developing countries have cited the need to produce improved early-maturing varieties (Cerna and Beaver, 1990). Large differences (50-250 d) in days to maturity are found in cultivated common bean (Singh, 1992). These differences are associated with differences in growth habit, degree of sensitivity to photoperiod and temperatures, and growing environments (Singh, 1992). Genetic control of earliness vs. lateness depends on prevailing day and night temperatures, photoperiod, and genotypes utilized in each study (Singh, 1991). Coyne and Mattson (1964) indicated that the photoperiodic response under long days was controlled primarily by qualitative genes. Later, Coyne (1966) observed polygenic control of this response under long days in two crosses, while some qualitative genes effects were observed in another cross. Under short days it appears that qualitative genes are not expressed and the variation is mainly due to the action of polygenes and environment. Coyne and Schuster (1974) found that early flowering and determinate growth habit were each determined by a single recessive gene with coupling linkage. Al-Mukhtar (1981) and Mohan (Singh, 1991) report monogenic control with complete dominance, whereas Leyna et al. (1982) report incomplete dominance in the F_1 generation for early flowering/maturity.

7.1.1.3 Horticultural attributes. Yield, maturity, and sieve size data alone are absolutely worthless unless related to quality. Since different end-product uses require different quality standards, it is necessary to know the requirements of each particular customer. An attempt has been made by Silbernagel and Drake (1978) to enable evaluations to standardize reporting of yield, maturity and sieve size data at the point of maximum yield and quality. Seed development can be useful because other quality factors, such as suture and pod wall fibre development, can be related to seed development. Quality also includes flavour, texture, carpel separation, skin sloughing, interlocular cavitation, internal tissue breakdown, and colour. These

factors can be evaluated later in product trials of the better lines that pass the preliminary evaluations based on simpler quality-screening techniques, such as the seed index (Silbernagel, 1986). Romanhernandez and Beaver (1996) provided a morphological marker for stage of development at harvest, which affects both the yield and quality of green-shelled beans. The appearance of the first dry pod was considered to be useful to begin the harvest of green-shelled beans because at least 85% of the estimated maximum pod yield had accumulated.

How techniques of breeding principles globalised in a snap bean breeding program is shown in the following paradigms:

1st paradigm: Prediction methods in early generation evaluation. Early crossing results are contradictory (see text). References from allogamous species may indicate how to choose parental lines. Handling of segregating populations, specific in maize (*Zea mays* L.), depends on the following assignments: (i) the broad generalization covering all the studies of the various types of gene action indicates that complete dominance is more important than overdominance in the crop improvement (Paterniani, 1973; Sprague and Eberhart, 1977; Jenkins, 1978); (ii) lines with general combining ability are mainly due to additive effects, whereas the components of variance for lines with specific combining ability are due to dominant effects (Sprague and Eberhart, 1977); and (iii) F_2 populations with tolerance to inbreeding are qualified (Hallauer, 1978). These imply that lines with general combining ability and crosses (F_1) with low inbreeding depression in F_2 have a desirable assemblage of genes that corresponds to segregating generations capable of developing elite lines (Koutsika-Sotiriou, 1999; Koutsika-Sotiriou and Karagounis, 2005).

2nd paradigm: Applying recurrent selection schemes. The recurrent selection aims, when applied in an autogamous species, such as snap bean, to generate improved sources of germplasm. The comparative advantages of recurrent selection over other methods, such as gamete (Singh, 1994; Singh et al., 1998), F_2-derived family (Urrea and Singh, 1994), and mass or pedigree (Singh et al., 1989a; Singh, 1995) selection are not yet obvious (Singh et al., 1999). However, recurrent selection cycle based on S_1 family yield would be the most appropriate and effective method for common bean improvement (Pandey and Gardner, 1992). Among seven recurrent selection methods, the S_1-progeny method has the highest estimated heritability percentage (78.8), proving its effectiveness (Lamkey and Hallauer, 1987) in maize.

3rd paradigm: Developing lines with tolerance to density. In soybean, optimum yields were achieved under a wider range of densities, from 3 to 50 plants/m^2 (Carpenter and Board, 1997a, b). Fasoula and Fasoula (2000) stated that the incorporation of genes for wider spectrum of optimum plant density may be attributed to an unconscious selection for higher yield per plant. The development of lines tolerant to density is essential for higher and stable yield per unit area (Fasoula and Fasoula, 2000; Tokatlidis et al., 2005). Snap bean varieties appear to be promising in improving for tolerance to density, owning to that they involve one major yield component (pod number). Thus, decreasing potential problems with yield component compensation observed in dry beans (Adams, 1967).

7.1.2 A Holistic Method for Developing Lines

As a holistic method for plant breeding, the honeycomb methodology was characterized. The honeycomb methodology was proposed by Fasoulas (1973, 1977) for the evaluation of quantitative traits of widely spaced single plants, grown under optimal field conditions (Bos, 1983; Robertson and Frey, 1987; Jensen, 1988; Borogevic, 1990; Sotiriou et al., 1996), combined with bulk or pedigree selection (Fasoulas, 1988). Honeycomb selection aims to eliminate deleterious genes and to exploit additive genetic effects prior to the exploitation of nonadditive effects. This approach should permit the accumulation of all the favourable genes in one genomic variant (Fasoulas, 1988).

Experimental designs, which are well adapted, when field plots as units of evaluation and selection are replaced by widely spaced single plants, are the honeycomb field designs (Fasoulas, 1973; Fasoulas and Fasoula, 1995). Honeycomb designs maximize efficiency in plant breeding by (i) preventing competition, thus identifying heritable superiority, and (ii) maximizing phenotypic expression and differentiation (Fasoulas, 1988, 1993). The honeycomb design of sowing (Fig. 1) is an attempt to solve the soil variability problems and is used for single plant and progeny selection. Every plant in the field occupies the centre of a complete replicate. This enables the comparison of each individual plant with surrounding plants representing all the remaining lines, including any controls. Evaluated plants are positioned in concentric rings, the size of which determines the size of the selection pressure applied. For example, when a plant in the centre of the hexagon out yields the surrounding six plants, it is selected by 14.3 percent intensity of selection. Since each plant is evaluated in relation to the hexagon of which it is the central plant, the hexagon is not fixed, but moving (Jensen, 1988; Borojevic, 1990), and this is what distinguishes the procedure from Gardner's (1961) grid system. Therefore, in a honeycomb design, the units of evaluation and selection at all stages of the breeding program are individual plants grown at the critical distance.

The concept of whole-genome phenotypic evaluation recognizes that genes controlling crop yield concern the genome as a whole and belong to three categories that correspond to the three components of yield potential: (1) genes that control yield ability per plant and expand the lower limit of the optimal plant density range; (2) genes that confer tolerance to biotic and abiotic stresses and expand the upper limit of the optimal plant density range; (3) genes that control variety responsiveness to inputs (Fasoula and Fasoula, 2002). The outcome of selection, based on whole-genome phenotypic evaluation during all generations of a breeding program, is high yielding, stable, and homeostatic (density-independent or density tolerant) varieties (Fasoula and Fasoula, 2002).

The honeycomb breeding was characterized as holistic for the following reasons: (i) it is based on individual plant performance, as a unit of evaluation and selection, instead of plot performance (Fasoulas 1973, 1977; Fasoulas and Fasoula, 1995); (ii) the genetic basis of high and stable crop yield delineate the conditions that provide selection efficiency in plant breeding (Fasoulas 1988; Fasoula and Fasoula, 2000); and (iii) genotypic level recognizing that genes controlling crop yield concern the

genome as a whole resulted in expressing yield potential components (Fasoula and Fasoula, 2000, 2002).

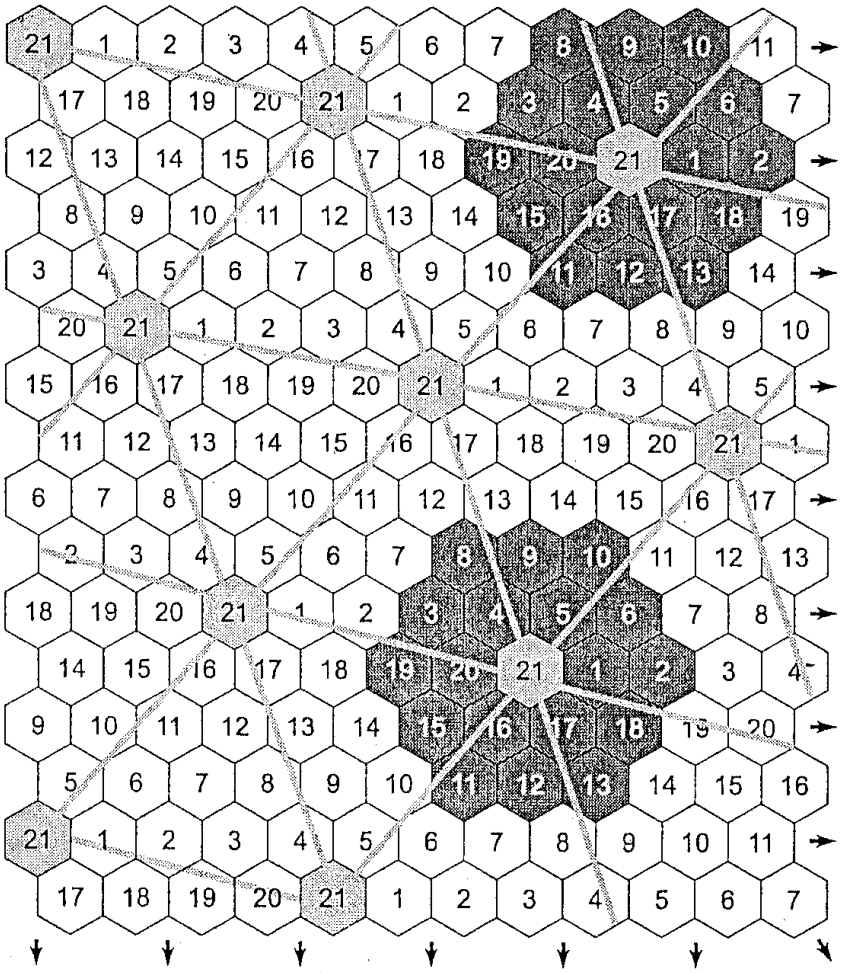

Fig. 1. The replicated R-21 honeycomb design evaluates a maximum of 21 entries. The moving replicate is exemplified by the darker shaded plots in the periphery of concentric circles whose central plot is entry 21 (Fasoulas and Fasoula, 1995).

As a positive remark in encouraging the application of honeycomb breeding in snap bean is Adams' (1967) work, which holds that common bean yield components, when controlled by genetically independent factors, develop in a sequential pattern. Developmentally induced associations occur among yield components when the yield components compete for limited nutrients or photosysnthates, thereby

preventing each component from achieving its genetic potential. A reduction or increase of one yield component would be compensated by an increase or decrease in another yield component. Such a buffered system results in yield stability due to the developmental plasticity of the yield components, and theoretically could limit the potential yield gains through the use of increased planting densities. Snap bean involves only one major yield component, the number of pods, and this decreasing potential problem with yield component compensation, was observed in dry beans.

Future prospects of honeycomb methodology to serve the developing horizons may be: (i) the organic breeding which demands scientific approaches and evaluation by the breeder (Lammerts van Bueren et al., 2003) on eco-breeding principles, such as the yield potential components (Koutsika-Sotiriou and Karagounis, 2005); and (ii) the challenge given by molecular breeding to study QTLs for traits relevant to plant breeding, where F_2's or backcrosses have to be raised as spaced plants in order to identify and score them for both markers and traits instead of plots (Kearsey and Luo, 2003).

Honeycomb breeding uncouples the reliable selection for yield and stability from the visual evaluation and offers the transition from single-trait evaluation to whole-genome phenotypic evaluation, applying the three components of crop yield potential (Fasoula and Fasoula, 2002):

7.1.2.1 Yield ability. The best selection criterion to estimate the yield per plant of a genotype is its mean yield per plant assessed in the absence of competition (isolation environment), when the breeder is focusing on genes that control yield ability (Fasoula and Fasoula, 2000). Selection in the absence of competition is effective in combining in autogamous-species the effective exploitation of additive genetic variation with the incorporation of genes ensuring greater tolerance to stresses (Fasoulas, 1997; Janick, 1999).

7.1.2.2 Resistance to stresses and genetic stability. Stability of performance is a complex trait, with a plethora of genes conferring resistance to both abiotic and biotic stresses, and interacting on many levels (Fasoula and Fasoula, 2000). Incorporation of genes conferring resistance to the multitude of biotic and abiotic stresses improves the genotype's individual buffering and by extension, its resistance to acquired differences that interfere with the equal sharing of resources and reduce yield (Fasoula and Fasoula, 2000). The spaced plant environment for selection (i.e. honeycomb designs), which was preferred to a competitive environment (Donald and Hamblin, 1976), enabled to calculate the coefficient of variability for any trait or yield (i.e. CV = standard deviation/mean) of individual plants, during the selection experiments. The CV is the most widely used parameter to quantify variability among individual plants of a crop stand (Edmeades and Daynard, 1979), and also is a way of estimating genetic yield improvement (Tollenaar and Wu, 1999). Under an isolation environment the smaller the CV of single plant yields of a particularly entry, the higher its tolerance to stresses and stability of performance. Also, one can quantify tolerance to stresses using a directly proportional criterion, the reciprocal of CV, i.e. the standardized entry mean (x/s). In this case, the larger the standardized

entry mean in the isolation environment, the higher the stability of performance in the crop environment.

7.1.2.3 Responsiveness to Inputs Ability. A review of the performance of widely grown varieties of past and recent eras (Evans, 1980; Duvick, 1992, 1996) revealed an essential component of the crop yield, which is the responsiveness to inputs (Fasoula and Fasoula 2000). That means, besides to genes responsible for yield and stability, a third category of genes exists, enabling crops to optimize productivity by responding to favourable growing conditions (Fasoula and Fasoula, 2000). It is therefore understandable that genotypes carrying genes for responsiveness to inputs will exploit improved growing conditions more effectively than entries derived from such genes. For measuring this ability, the genotype's selection differential is converted into standardized units, i.e. into the standardized selection differential (SSD = xsel-x)/s), that is more reliable and quantifies the responsiveness to inputs ability (Fasoula and Fasoula, 2002). The responsiveness to inputs needs some prerequisites to be estimated, such as intensity of selection and selection scheme that was applied.

How techniques of honeycomb breeding were applied in a snap bean breeding program is shown in the following paradigms:

1st paradigm: Defining the End-Target. Aiming to restore or even improve a snap bean variety, an intra-selection breeding program was applied (Traka-Mavrona et al., 2000). The program started with the study of the existing genetic variability for early maturity and pod yield potential, based on widely spaced plant performance for the prementioned traits. For this purpose, estimating existing genetic variability in the variety was considered the technique for defining the end-target. Examining the single-plant frequency distributions of source material for number of pods per plant, in three subsequent dates, the data showed that: (i) for early maturity, with either strong positive skewness (Fig. 2) or positive skewness (Fig. 3), the frequency of unfavourable alleles was high; and (ii) for total yield per plant, with a normal distribution (Fig. 4), the genetic variability was mainly consisting of additive genes and remained in equilibrium. The diagnostic role of frequency distributions of source material showed that the end-target should be selection for early maturity, keeping and stabilizing high yield.

2nd paradigm: Selection Process. The honeycomb pedigree intra-selection breeding program was applied in a snap bean variety starting with the target of earliness and yield stability, and progressively advancing with the target of seed shape uniformity. The experiments followed the replicated honeycomb design R-21 (Fasoulas, 1973; Fasoulas and Fasoula, 1995), consisting of 21 selected families, which were examined for earliness, yield potential and/or seed shape, and analyzed by the Batzios and Roupakias (1997) microcomputer program. The average response to selection was 2.43-3.15 and 0.13-0.42 pods/plant per generation for earliness and yield, respectively. The coefficient of variability (CV) of earliness decreased from 81.33 to 39.43% and for yield was stabilized at the end-value of almost 28%. All selections produced high and stable early fresh pod harvest even 53 days after planting, while the control was still at the vegetative phase. The yield of selected

progenies was 219-242% superior compared with source material. Also, seed stocks of all selections were of the normal long shape (Traka-Mavrona et al., 2000, 2001).

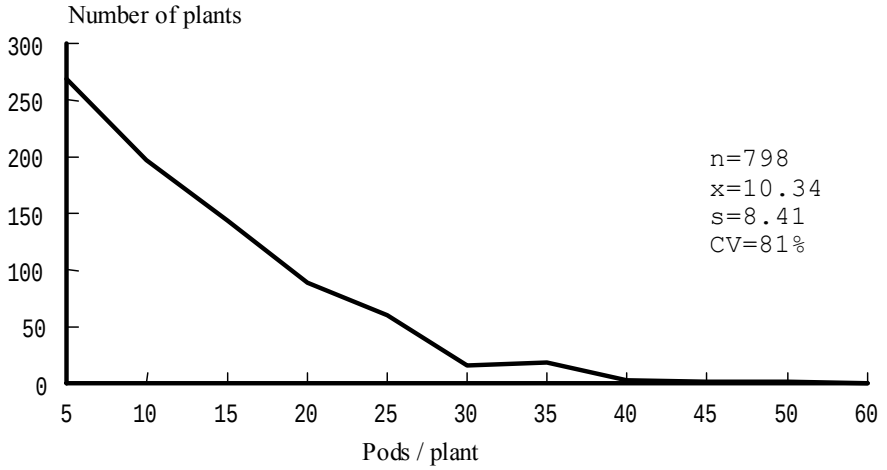

Fig. 2. Single-plant distribution of early maturing pods of source material 70 days after planting (Traka-Mavrona, et al., 2000).

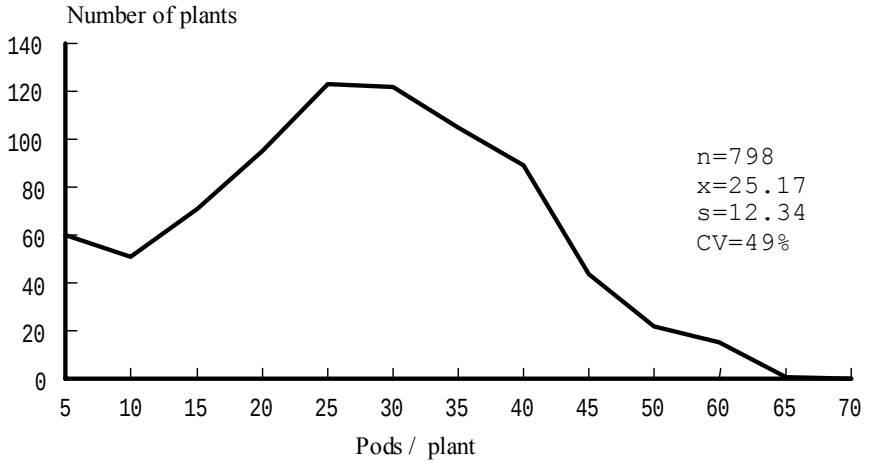

Fig. 3. Single-plant distribution of the number of pods of source material 78 days after planting (Traka-Mavrona et al., 2000).

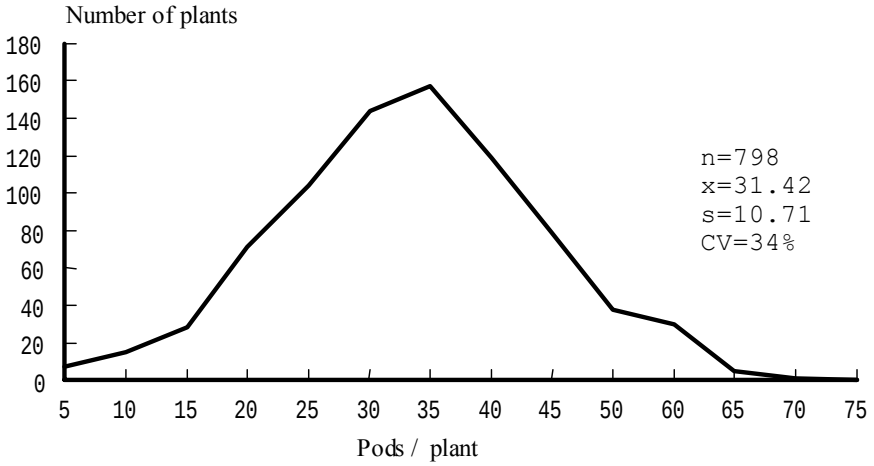

Fig. 4. Single-plant distribution of total number of pods of source material 92 days after planting (Traka-Mavrona et al., 2000).

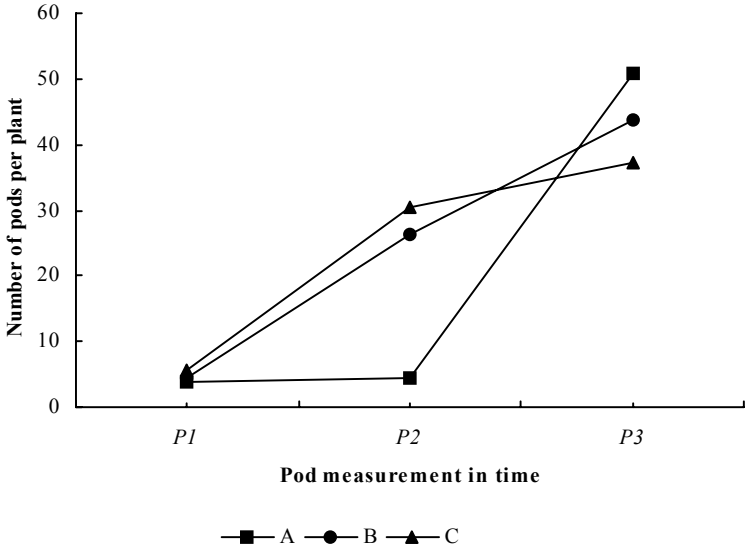

Fig. 5. Variation in the number of pods of each measurement in time of the source variety as affected by the planting date. A, B, C = the three planting dates. P1, P2, P3 = the three pod measurements (Traka-Mavrona et al., 2002b).

3rd paradigm: Handling Abiotic Stresses. Manipulations for decreasing the magnitude of the environment-dependent performance of a climbing type snap bean variety by exposing the variety to abiotic diversity, i.e. planting on three dates for two successive years, was the aim of a breeding program. For assessing the agronomic stability of the variety, three statistical techniques were simultaneously followed: (i) partition of variance, which defined a significant planting date X pod measurement interaction (Fig. 5); (ii) regression analysis, which showed that temperature accumulation for each environment was highly correlated (r=0.97) with pod yield; and (iii) pod-yield frequency distributions of space-planted single plants, which showed positive skewness for all measurements, while the high coefficient of variability (CV) values quantified the genotype X environment interaction (Fig. 6). The pre-breeding manipulations resulted in gaining pod yield stability (Traka-Mavrona et al., 2002b).

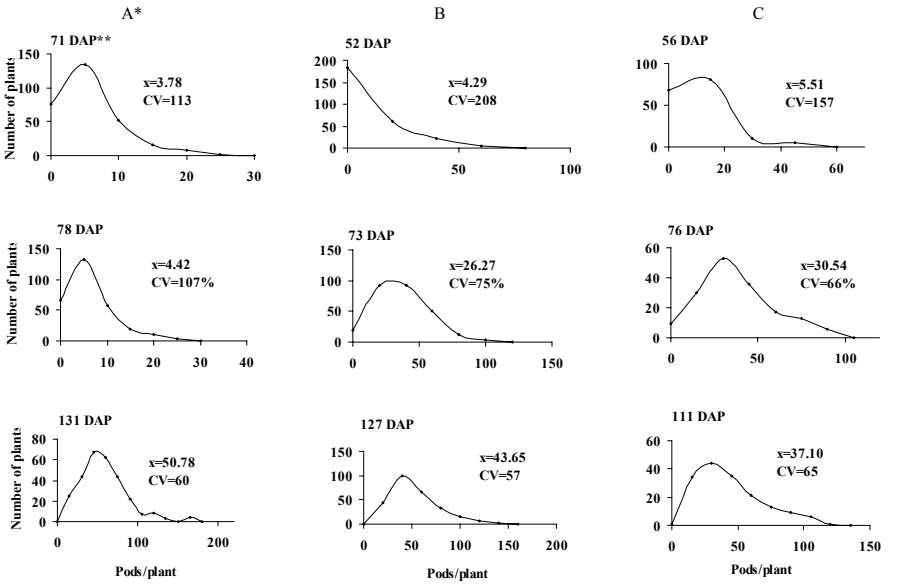

Fig. 6. Single-plant frequency distributions of first (upper), second (centre) and third (below) pod measurement of the source variety at each of the growing environments (planting dates). *A (left), *B (centre), *C (right) = the three planting dates, **DAP = days after planting (Traka-Mavrona et al., 2002b).

8 Integration of New Biotechnologies in Breeding Programs

Molecular techniques are radically altering the way that plant breeding is being performed. What has changed however is the scale at which genes can be sequenced, their expression can be analyzed, and proteins can be identified. Genomics, transcriptomics, and proteomics allow the study of genes of a particular organism. When all these applied to beans, the name Phaseomics is formed which is an international consortium that aims at establishing the necessary framework of knowledge and materials for an integrated approach of new biotechnologies in breeding programs (Broughton et al., 2003). The genome size of *Phaseolus* is 637 Mbp/1C, one of the smallest among legumes (Zhang et al., 1996). Only a very limited number of *Phaseolus* repeats have been isolated and characterized (Garber et al., 1999). Identification of individual chromosomes is difficult due to similar morphology and lack of distinct chromosomal landmarks (Broughton et al., 2003).

The basic DNA sequence is the most important information, necessary to address fundamental and applied questions in the agricultural science. Although this information is complete for species (e.g. *Arabidopsis thaliana* and *Oryza sativa* L.), public databases hold relatively few entries for *Phaseolus* (< 500 nuclear-encoded genes). There are several ways for obtaining molecular markers inter-alia, one is the sequence messenger RNA's extracted from tissues of interest (e.g. developing pods), and the other is to isolate the mRNA (and hence prepare a cDNA library). Both pre-described ways constitute an efficient way of generating data that can interchangeably be applied in traditional breeding programs or completion of genome's sequence (Broughton et al., 2003). Taking into account the innovative approach to common bean, the steps and prospects of novel processes are outlined as follows:

8.1 Marker-Assisted Selection

Molecular information can be used in several ways to make the plant breeding process more efficient, mainly with so-called marker-assisted selection (MAS) schemes (Dekkers and Hospital, 2002). Selection decision in breeding programs can be based on phenotypic information alone (conventional selection), on molecular information alone, or on a combination of both. Herein, breeding strategies involving selection based on molecular information alone may reduce the selection phase. Based on phenotype and/or molecular information available prior to the start of the selection program, the breeder defines the ideal genotype (ideotype) at a collection of loci that allows production of the ideotype most rapidly, based simply on DNA analysis (marker genotypes). Finally, on completion of the MAS program phenotypic evaluation is carried out in order to evaluate the agronomic value of the resulting progenies (Hospital, 2003).

Below, the aforementioned data are presented in the form of current examples: A random amplified polymorphic DNA (RAPD) marker directly linked with a resistance gene was identified in a snap bean recombinant inbred population consisting of 94 F_3-F_7 recombinant inbred lines that had uniform segregation for disease reaction to BCTV. Results showed that sequence characterized amplified

region (SCAR) would be highly useful for marker-assisted selection of *Bct* in snap and dry bean originating from the Andean gene pool (Larsen and Miklas, 2003). In addition to *Bct* resistance, to BGMV resistances, a major gene for resistance to anthracnose (*Colletotrichum lindemuthianum*), and QTL for resistance to common bacterial blight (*Xanthomonas axonopodis phaseoli*), white mold (*Sclerotinia sclerotiorum*), and ashy stem blight (*Macrophomina phaseolina*) mapped in the same region of the chromosome B7 (Kelly et al., 2003), which further supports the presence of a resistance gene cluster in this region of chromosome B7. *Bct* is located approximately 25 cM from the *P* locus that conditions seed coat colour (recessive *p* gives white seed colour) (Larsen and Miklas, 2003). Ten random amplified polymorphic DNA (RAPD) markers linked to genes for five classical marker traits: dark green savoy leaf (*dgs*), blue flower (*blu*), silvery green pod (*arg*), yellow wax pod (*y*), and flat pod (a spontaneous mutation from round to flat pod in "Hialeah" snap bean) can be integrated to form a more complete and informative genetic linkage map (Beltran et al., 2002). Bean populations with improved ability to germinate in low temperature are being used to identify regions of the genome associated with this trait. The results will allow to immediately implement MAS in the breeding program of Canada (Broughton et al., 2003), aimed to lengthen the growing season, in order to introgress this trait.

8.2 Genomic Colinearity

Over the past 15 years it has become apparent that the organization of sets of orthologous genes within related genomes has been conserved. This phenomenon of conservation of gene order is often referred to as synteny or colinearity. It refers to conservation of gene content, order and orientation between chromosomes of different species or between non-homologous chromosomes within a single species (Newbury and Paterson, 2003). The colinearity based on genetic mapping has been termed "macrosynteny", while that based on gene order "miscrosynteny" (Newbury and Paterson, 2003). Boutin et al. (1995) revealed the existence of high degree of colinearity between mungbean (*V. radiata*) and common bean linkage maps; comparisons between these linkage groups and that of soybean showed that short sections of the soybean linkage map exhibited colinearity. Humphry et al. (2002) established a high level of colinearity between the linkage maps of lablab (*Lablab purpureus*) and mungbean. Lee et al. (2001) examined homologous regions of the genomes of soybean, common bean, and mungbean and showed that there is not only conservation of large regions of the genomes but these conserved linkage blocks are also represented twice in the soybean genome. In addition there is also reported that genomic regions showing conserved gene order in three legume genomes, are also relatively conserved in *Arabidopsis* (Lee et al., 2001).

There are several direct benefits of knowledge of colinearity relationships between plant species for those involved with crop improvement (Newbury and Paterson, 2003): (1) It allows the prediction of the location of genes controlling a particular function, i.e. a map-based cloning of a plant disease resistance gene. For instance, over 500 markers, including AFLPs, micro-satellites, RAPDs and RFLPs, are mapped and used to tag quantitative trait loci (QTL) for characteristics of interest

to plant breeders. These include abiotic stress tolerance (low phosphorus, aluminium toxicity, and drought tolerance), micronutrient content (iron and zinc), as well as insect and disease resistance (Broughton et al., 2003). (2) It improves prospects of the transfer of such traits via homologous recombination in wide crosses, i.e. mapping of *P. filiformis* and *P. angustissimus* enable the identification of the introgressed segments in the inter-specific hybrids. Furthermore, the gene map will be used to examine micro-synteny of *Phaseolus* in comparison with other species (Bett et al., Broughton et al., 2003). (3) It provides markers that can be transferred from a well-mapped to a less well-studied species; i.e. genomic interspecies micro-array hybridization will identify genes that are expressed in *P. vulgaris*, and provide a quantitative relationship assessment of the relationships between beans, soybeans and *M. truncatula* (Broughton et al., 2003). *M. truncatula* is becoming established as a model legume species because of its small genome (Cook, 1999). (4) It serves as a catalogue of genes that, in the future, could be transferred by genetic engineering in order to fine-tune aspects of performance.

8.3 Genomics

The analysis and development of quantitative trait loci (QTLs) has an enormous importance in breeding programs. Although it is possible to undertake breeding programs using only phenotypic selection, an understanding of the number and location of QTLs controlling performance for a target trait can markedly enhance the efficiency of breeding. Two main marker types have been emphasized: sequence characterized amplified region (SCAR) markers and micro-satellites or simple sequence repeats (SSRs). These markers have been essential for mapping and tagging genes of agronomic importance and for their eventual selection in marker-based breeding schemes. Other marker systems, such as AFLPs and RAPDs have been used to study the diversity within different species of the genus *Phaseolus* and of the many accessions that are stored in the germplasm bank (Broughton et al., 2003).

Complex inheritance patterns and strong environmental effects may limit the value of phenotypic estimates of traits and the efficiency of a bean breeding program. Furthermore, inverse correlations among agronomic traits may hinder the progress of plant improvement. The use of molecular markers should improve the understanding of the genetic factors and is expected to assist in the selection of superior genotypes. Molecular markers are an efficient method for determining genetic relationships among various types of germplasm, as they are not affected by environmental or epistatic interactions that may affect morphological traits. However, the use of molecular markers to select families and populations that are harvested in bulks in early segregating generations (F_2 to F_3) may present a prohibitive cost for screening a large number of plants in each generation (Singh, 2001). Several studies have been reported on cultivated and wild common beans using different kinds of molecular markers. Some of them have shown that diversity was greater in wild populations than in cultivated beans (Gepts, 1990; Sonnante et al., 1994), while other studies did not reveal any difference (Singh et al., 1991a, b; Becerra-Velasquez and Gepts, 1994; Cattan-Toupance et al., 1998).

Three cDNA libraries have been made from bean tissues at CIAT. The first was a leaf cDNA library constructed from total mRNA extracted from leaves of adult plants of the Andean variety G19833. The source genotype is tolerant to low phosphorus levels in soils and has multiple disease resistance including anthracnose, angular leaf spot and Ascochyta leaf blight. Two root cDNA libraries have also been made from mRNA extracted from adventitious and basal roots grown under phosphorus deficiency stress for the same genotype (Broughton et al., 2003). About 4000 clones have been sequenced so far and the expressed sequence-tags (ESTs) are being used to develop molecular markers. To put our argument in a more generalized manner some markers which were determined are: QTL for common bacterial blight resistance (CBB), and for seed yield, yield components, plant architecture (Tar'an et al., 1998, 2002), several morphological traits; and molecular markers for leaf-hopper (*Empoasca fabae*) resistance loci based on resistant and susceptible lines (Broughton et al., 2003). The European *Phaseolus* database was established on the initiative of the European Cooperative Program for Crop Genetic Resources Networks (ECR/GR) at Linz, Austria (www.genebank.at). The structure of the database follows the principles of the FAO/IPGRI Multi-Crop Passport Descriptors list.

8.4 Genetic Transformation and In Vitro Regeneration

Gene transfer techniques offer new possibilities for bean improvement. Biolistic and *Agrobacterium tumefaciens*-mediated transformation strategies are being tested for transformation. Bean regeneration via organogenesis from apical meristems transformed by the biolistic method was obtained by Aragao and Rech (1997). Entirely open shoot meristems that are approachable by gene coated particles are a prerequisite for efficient gene transfer into regenerated cells (Veltcheva et al., 2005). Klu (1997) regenerated bean plants via direct organogenesis from cotyledonary modal tissue. Histological studies revealed that buds-developed from sub-epidermal cells of the node, confirming the adventitious nature of these structures (Veltcheva et al., 2005), obtained plant regeneration from very immature embryos of *P. vulgaris*. Thus far, no protocol for successful plant regeneration from protoplast-derived calli has been published, while a successful regeneration system of anther-derived callus was developed (Veltcheva et al., 2005).

The rate of transformation of *P. vulgaris* is extremely low, only 0.02% of the regenerated plants transmit the introduced DNA to their progeny (Broughton et al., 2003). Common bean has been found susceptible to *Agrobacterium* wild-type strains and derivatives (Lewis and Bliss, 1994). At CIAT through congruity backcross and embryo rescue methods, inter-specific hybrids with *P. acutifolius* transformed with the biolistic method have been developed (Broughton et al., 2003). Also, an improved *P. acutifolius* agrobacterium based transformation has developed, aimed to introduce a high-level accumulation of modified arcelin (seed storage protein) improving nutritional balance. Ten lines from transformed *P. acutifolius* were generated (De Clercq et al., 2002).

Common bean can regenerate in vitro at low efficiency either indirectly (through callus stage) or directly (through somatic embryogenesis and organogenesis). Bean varieties carrying modified genetic make-up as a result of either gene transformation

or inter-specific crosses and subsequent embryo rescue were produced. Elite varieties will be the parents of the next generation of improved genotypes by involvement in conventional breeding programs (Veltcheva et al., 2005).

8.5 Genomics Meets Bioinformatics

The utilization and management of genetic resources have been revolutionized by major advances in molecular biology and information technology. Molecular biology has allowed the use of DNA markers and DNA sequencing for better description and manipulation of genetic diversity, and the cloning and transgenic manipulation of traits. Information technology has allowed an explosion of gene sequence and trait data, and the international access to databases through the internet. Some of the ways in which molecular techniques and information technology have led to improved management and utilization of genetic resources are (Godwin, 2003): (i) better description and measurement of diversity, (ii) better screening of variation for a trait, and (iii) better management of data sets.

PhaseoBase is a central database of all data generated by the consortium "Phaseomics", and will be generated and made ready for mining by the consortium members. This database will be modelled on the XGI/ISYS system of the US-based National Centre for Genomic Research (NCGR) (Broughton et al., 2003).

Common bean is essential to understand how to develop new traits, especially those of agronomic interest. Two main subjects connected with evolutionary pattern are studied (Gepts, Broughton et al., 2003): (i) evolution of small multi-gene families involved in seed protein production, i.e. phaseolin and the APA family, i.e. the arcelin-phytohaemogglutinin-alpha-amylase inhibitor family that is involved in defence against animal predators, especially seed weevils, and (ii) evolution of domestication traits including those that distinguish various bean varieties from one another. As examples, the determinacy gene controls growth habit, which is often found in domestication bush beans, especially in snap bean varieties, where it assures both earliness and once-over destructive harvest of pods of more or less at the same age. The pod-scattering gene is essential to wild beans to assure seed dissemination and reproduction of the plant. In domesticated beans, this is obviously a deleterious trait.

Genomics offers the potential to isolate the genes responsible for these traits and, in turn, improve them in superior varieties. In addition, the determinacy (*fin*) and pod string (*st*) loci have not yet been isolated. In the last few years, great progress has been made in identifying the molecular mechanisms underlying reduction of grain shattering, which involved genetic loci of large effect (Ferrandiz, 2002). Li et al. (2006) undermined the gene function necessary for the normal development of an abscission layer that controls the separation of a grain from the pedicel in rice. These genes could also be used to identify and characterize naturally occurring genetic variation in the form of QTLs affecting dehiscence. Since completely indehiscent makes seed harvesting more difficult, QTLs of moderate effect may represent more useful tools for the line tuning the dehiscence process (Dinnery and Yanofsky, 2004). An additional tool, which will have great repercussions in crop diversity, characterization and utilization, is linkage disequilibrium (LD) analysis. Linkage

disequilibrium analysis is an alternative measure of association, which relies on existing populations of unrelated individuals rather than on segregating populations resulted from a cross (Broughton et al., 2003).

9 Seed Production

The rapid increase of seed stocks of new and improved varieties is essential to the success of modern agriculture. The production of adequate seed of new varieties is based on an efficient seed production system. To be effective, such a system, two assumptions have been made: (i) the development of the variety is the primary function of the breeder; and (ii) the increase and the distribution can be handled most expeditiously by seed producers. Much of the detail in distribution of seed of varieties was developed through publicly or privately supported breeding programs.

9.1 How a New Variety is Accepted

A new vegetable variety shall be accepted for the production of basic or certified seed only when a designated authority has checked that it is distinct and that its generation used for vegetable production has sufficiently uniform and stable characters. According to article 7 of the 1961/1972 and 1978 acts and article 12 of the 1991 act of the International Union for the Protection of new Varieties of Plants (UPOV), protection can only be granted in respect of a new plant variety after examination of the variety has shown that it complies with the requirements for protection laid down in those acts and, in particular, that the variety is distinct (D) from any other variety and that it is sufficient uniform (U) and stable (S), or "DUS" in short. The examination, or "DUS" test, is based mainly on growing tests, carried out by the authority competent for granting plant breeders' rights or by separate institutions, or, in some cases, on the basis of growing tests carried out by the breeder. The examination generates a description of the variety, using its relevant morphological or physiological characteristics, by which it can be defined as a variety in terms of article 1 of the 1991 act of the convention of UPOV. In each country an official national list of varieties that have been accepted are published, and annually revised. Synonyms and homonyms are clearly indicated in these lists. Only seed of listed varieties is eligible for certification. The name and address of the maintainer of each variety is given.

In summary, the general principles for the conduct of "DUS" tests, as summarized in the UPOV (1995) guidelines for snap bean, are as follows: (i) the seed should meet the minimum requirements for germination capacity, moisture content and purity; (ii) the minimum duration of tests should be two growing periods; (iii) the tests should be carried out under conditions ensuring normal growth; (iv) as a minimum each test should include a total of 150 plants for dwarf beans and 60 plants for climbing beans which should be divided between two or more replicates; and (v) all observations should be made on 20 plants. To facilitate the assessment of distinctness the collection of snap bean varieties to be grown should be divided into groups according to plant growth type, shape of pod cross section, pod ground colour, pod stringiness and number of seed colours. In total, 47 characteristics are listed to assess distinctness, uniformity and stability of snap bean

varieties, out of which 21 should be used on all varieties, in every growing period and always be included in the variety descriptions. In particular, the following characteristics should be included in the variety descriptions: plant: growth type; leaf: green colour; flower: size of bract, colour of standard, colour of wing; pod: length, shape of cross section, ground colour, secondary colour, hue of secondary colour, stringiness, length of beak; seed: weight, shape of median longitudinal section, number of colours, main colour, predominant secondary colour, colour of hilar ring; time of flowering; resistance to bean anthracnose; resistance to halo blight.

9.2 Classes of Certified Seed

The main purpose of seed certification is to maintain new and improved varieties as developed and described by plant breeders. Use of certified seed, whenever available, is one assurance of obtaining seed accurately labelled for purity and quality. It is not an assurance of obtaining an adapted variety, unless the variety has tested and has been found to be suitable for production in the area where the buyer expects to plant the seed.

Three classes of seed are recognized by seed certification agencies (Poehlman and Sleper, 1995; OECD, 2000):

(i) Breeder or pre-basic seed is seed directly produced or controlled by the originating plant breeder or institution. Breeder seed provides the source for the increase of foundation seed.

(ii) Basic or foundation seed means seed which has been produced under the responsibility of the breeder according to accepted practices for the maintenance of the genetic identity and purity of the variety. Basic seed is the source of certified seed.

(iii) Certified seed means seed which is produced directly from basic seed or from seed of a generation prior to basic seed. Certified seed is subjected to post-control tests.

Specifically for vegetable seed, the class of standard seed is additionally designated (OECD, 2000). Standard seed is of sufficient varietal identity and purity, which is declared by the supplier as being true to the variety and of satisfactory varietal purity, uniformity and stability. Standard seed is subject to official post-control by check inspection to verify its varietal identity and purity.

According to Silbernagel et al. (1991), the maintenance of pure basic seed lots is a highly specialized task requiring elaborate monitoring, roguing, and careful separation of different seed lots, whereas breeders' seed of most snap bean varieties must be reselected anew every two or three years. Several hundred single plant selections are made and they are single-row planted in the following generation. Each row is checked carefully for off-types, which are then discarded. Selections which are uniformly indistinguishable from the variety standard description are bulked, carefully monitored, and rogued free of off-types in the next one or two generations of seed multiplication. At best, that seed lot can be reproduced for sales purposes (with monitoring only) for one or two generations before it has to be replaced. This maintenance of basic seed stocks free of off-types is a highly specialized art that requires elaborate monitoring, roguing, and scrupulous separating

of seed stocks. For these purposes, harvest machinery and containers have to be meticulously cleaned. The complete process requires a highly skilled and stable labour force. Important off-types are the flat or oval podded and stringy podded rogues, which create the greatest havoc in the processing plants and generate the greatest number of customer complaints. The genes responsible for pod shape and fibre appear to be less stable than other genes, and have a higher reversion rate to high fibre types (Atkin, 1972; Atkin and Robinson, Myers and Baggett, 1999). Seed companies spend a lot of time and effort trying to reduce the frequency of this defect, by roguing (physically removing off-type plants during the growing season), single-plant selection, and mechanical precision sizing. Most have established tolerance limits for each stage of seed production increase, aimed at providing the processor with less than 2% flats in the final processing crop. At the breeder's seed stage, a maximum of eight plants per thousand is a good rule of thumb. Seedlots of most varieties have to be replaced every 3-5 years, to keep the frequency of flats and string mutants within acceptable limits.

9.3 How a Variety is Certified

In general, certification involves the following steps (Parsons, 1985; Poehlman and Sleper, 1995):

(1) The grower must plant basic seed of an approved variety.

(2) The seed must be planted on clean ground. The field should not have been planted in the previous year to another variety of the same crop, or to other crops that might volunteer and affect the purity of the crop being certified. Noxious weeds are removed before harvest, and borders are clipped where necessary to maintain seed purity.

(3) In cross-pollinated crops, isolation of the seed-producing field is required. Although common bean is an autogamous species, extremely high rates of natural outcrossing in some environments and genotypes have been reported (Wells et al., 1988).

(4) Off-type plants and mixtures are rogued by the grower before harvest, or before flowering.

(5) Field inspections are made by representatives of the seed-certifying agency to check on the purity of the variety.

(6) Seed inspections are made by representatives of the seed improvement association as necessary to observe and supervise the harvesting, conditioning, bagging, and other processing operations.

(7) Official tags supplied by the seed-certifying agency are sealed on the bags of seed approved for certification.

The minimum requirements for the production of basic and certified seed under the Organization for Economic Cooperation and Development vegetable seed scheme (OECD, 2000) are as follows:

(1) Health of seed used for seed crop production. The seed used for seed crop production should be as pest and disease free as possible. Its health should be checked before use and, if pest or disease organisms against which there is an effective seed treatment are present, that treatment should be applied.

(2) Previous cropping. Seed production fields or glasshouses shall be sufficiently free from volunteer plants to avoid contamination of the crop seed by: (i) any seed which is difficult to remove from the crop seed; (ii) cross-pollination; (iii) seed-borne diseases transmitted from volunteer plants. The previous cropping shall be such that there is the least possible risk of any soil-borne diseases being present which could subsequently be transmitted in the harvested seed. If any previous crops could have made the fields or glasshouses unsuitable for the above reasons, adequate measures must be taken.

(3) Isolation. Seed crops shall be isolated from all sources of pollen contamination and seed-borne diseases including seed-borne virus infection and wild plants that might serve as a source of disease.

(4) Field inspection. Each crop of basic seed shall be inspected at least once at an appropriate stage or stages of growth on behalf of the designated authority by inspectors and, in their inspections, responsible only to the designated authority. At least 20% of the crops of certified seed of each species shall be inspected by these inspectors.

Seed stocks of snap bean must be treated for control of plant disease and pest, and also to improve crop stand (Harman, 1991; Paulitz, 1992; Keinath et al., 2000; Elliott et al., 2001). They must have a high level of viability, have uniform emergence and early seedling vigour, and be capable of long-term storage (2-3 years) without serious deterioration. Variable seedling emergence and vigour can result from inherent genetic characteristics or from improper seed harvest, storage, and/or handling conditions (Silbernagel and Bruke; Copeland, Silbernagel, 1986). Single-plant selections and small bulks can also be compared for seed yield and quality (White and Gonzalez, 1990; Gonzalez et al., 2006). Those lines with comparatively low yield, highly variable seed size and shape, shrunken poorly developed seed, or a high proportion (>2%) of seed coat rupture should be categorically discarded, providing there is no other overwhelming reason to keep a particular line (Silbernagel, 1986). The remaining lines are then given Dickson's (1975), and Dickson and Boettger (Silbernagel 1986) nick test for tightness of seed coat adherence, the frequency of transverse cotyledon cracks, and thickness of the seed coat. Next, the best candidate lines are given a seed test for rate of water imbibition as recommended by Dickson and Boettger (Silbernagel, 1986). They found a too-rapid rate of water uptake to be correlated with poor stands and weak seedlings and suggested elimination of both problems by selection of semihard seed. Resistance to mechanical damage is also rated in dried lots of seed (6% compared to a 14% moisture control lot, fresh-weight basis) dropped several times onto an inclined steel plate from about 2 m (Dickson and Boettger, Silbernagel, 1986). The smaller the seed quality difference between dropped and not-dropped seed of the two moisture levels, the more tolerant the line is to mechanical injury. Seed damage can be estimated by comparing the percentage of broken seed and the percentage of hairline cracks found via the water test or by standard germination tests. The seed coat crack (water) test is done with several replications of 100 apparently sound seeds placed in water at room temperature. After 2-3 min, those with hairline seed coat cracks wrinkle in the vicinity of the crack. Sound seed takes much longer to begin imbibition through the micropyles or hilum (Kyle and Randall, 1963).

Breeders might consider selection for plant and pod characteristics that indirectly lead to improved seed quality. Westermann and Clothers (1977) showed that snap beans grown for seed also respond to high-density culture. Silbernagel (Silbernagel, 1986) suggested that direct harvesting of snap bean seed grown under high-density culture would eliminate many of the problems contributing to decreased seed quality that are associated with the present windrow system. Windrowed beans are cut below the soil line and laid on the soil surface to dry, where they may be exposed to moisture, causing moulds following rains, or subsequent over drying. The rubber-belt thresher proposed by Silbernagel strips dry pods from standing mature plants. The rubber belts extract seed with a minimum of mechanical damage; and since the plants are not windrowed, there is less seed spoilage from stains and moulds during rainy weather. To facilitate optimization of the system for high-density culture followed by direct seed harvest, breeders should select for a very concentrated pod maturity, numerous small vertically oriented leaves, and a strong, upright, narrow plant habit.

The standard germination test (Ellis et al., 1985a, b) consists of several hundred seeds in wet sand (20% moisture), perlite, or vermiculite, or in rolled paper towels at about 21 0C. After 7 days, those seedlings with the equivalent of at least one sound primary leaf, one cotyledon, a normal shoot and root tip, and that are at least half the normal size, are counted as germinated. If more detailed information is required, the seedlings can be classified as to the percentage of healthy, vigorous, normal seedlings (HVN) (Silbernagel, Silbernagel, 1986). Then the product of percentage emergence multiplied by percentage HVN seedlings is used to develop a seed quality estimate (SQE). Lines can be even more critically evaluated by the seed quality index (SQI), which is the product of the seed emergence index (percentage emergence X rate of emergence) X the percentage of perfect seedlings (Silbernagel, Silbernagel, 1986).

9.4 Additional Remarks on Snap Bean Seed Production

In any seed production system maintaining variety purity is important. Through outcrossing, mechanical mixtures, and spontaneous mutation, genetic variation creeps into seed lots. Seedsmen incur significant expense in walking fields during the growing season to rogue off-types, and in putting the seed through elaborate milling systems to eliminate off-types at the seed level. Snap bean seed is more difficult to produce because of sensitivity to injury during harvest, conditioning, planting, and germination, and reduced resistance to cold stress and soil-borne pathogens than dry bean seed (Myers and Baggett, 1999). Certain steps can be taken to reduce the chances of mechanical injury, such as avoiding drops over 50 cm and stabilizing seed moisture at around 12% (Dickson and Boettger, Myers and Baggett, 1999; Taylor and Dickson, 1987). Special equipment is required for harvest and conditioning, and special techniques such as priming allow seedsmen to produce high quality seed. Both the harvest and milling equipment and the varieties can be improved. Breeders can develop tougher seed as detailed above by selecting for resistance to mechanical and imbibitional damage.

Among vegetable crops, snap beans require great time and effort to maintain purity. One reason for this is that the harvest comes in the form of pods instead of seed, which complicates the maintenance procedure (Silbernagel et al., 1991). The maintenance of a variety encloses some obligations for the breeder. During multiplication, there are four requirements placed upon the breeder: purity, quality, health, and uniformity (Simmonds, 1979). The first three are basically the requirements of certification, while the fourth, uniformity, is a special requirement placed upon the breeder by usage. In meeting the three first requirements, the breeder is simply doing what the commercial multiplier will do later, but he is doing it at an extremely high level. The effects of a mistake or bad luck will inevitably be increased during subsequent multiplication of the variety. Usually, the maintenance of a variety is performed by discarding off types and once-over harvesting the dry seed production. However, sometimes diversions from the original type of a variety related to morphological abnormalities may be appeared. Sources of genetic variation in a self-pollinated crop, such as bean, may be varieties' heterogeneity, mutations, or insect-cross pollination (Silbernagel et al., 1991). Pearson (1956) and Riley et al. (Tokatlidis et al., 1998) reported in wheat (*Triticum aestivum* L.) that homozygosity enhances the frequency of chiasma formation and recombination. The maintenance, especially, of local varieties encloses an essential role of the plant breeder, the saving of the seed nowadays. This role should not permit the downgrade of local varieties in their main characters that render them competitive in the market. Large collections of snap bean types have been amassed as an urgent necessity for the use of plant breeders and as a protection against genetic erosion. A methodology of widely spaced single-plant combined pedigree intraselection was proposed, which can be applied in a repetitive way, in time defined by the breeder (Traka-Mavrona et al., 2000, 2002a, 2003). This methodology contributed to monitor two snap bean varieties' deviations that were realized as late maturity, unstable yield, and appearance of certain pod and seed abnormalities, and finally removed the deviations, saving for the breeder the valuable adapted varieties (Traka-Mavrona et al., 2000, 2001, 2002a, 2003). From a practical point of view, to see that highly deviant material can be easily detected in variety maintenance seed stocks, random amplified (RAPD) markers were used to follow changes occurring at the molecular level. The results showed that the changes that occurred during the selection process also could be followed by molecular marker polymorphism detection throughout the process, supporting the suggestion that molecular tools could serve to study the problem of off-type rogues and also predict the efficiency of further selection based on a particular breeding methodology (Tertivanidis et al., 2003).

Beans are the most important grain legumes for direct human consumption in the world (Broughton et al., 2003). Many local varieties are grown in Latin America and Africa, which are inferior in quality compared to commercial varieties but have a greater degree of horizontal disease resistance (Grubben, 1977). Social factors and ecological constraints determine weather beans are grown in a particular region. As agriculture and social systems have evolved together, the current state of farming systems is the result of the interaction of climate, edaphic, biotic, and social factors. Climbing varieties of snap bean are popular in home gardens and winter sowings in relatively warmer regions or greenhouses since they produce pods over a longer

period than the bushy varieties, which are more generally grown on a commercial scale. Western seed companies produce seed of the bush type snap bean varieties in the upland areas of the East Africa, which have a suitable warm-temperate climate. Snap bean is widely grown for supplying the markets of large cities due to the extremely high prices paid by the public. The importance of cut-of-season snap beans grown in Africa (Senegal, Kenya) for export to Europe is increasing, although more recently subject to fluctuation in demand, due to the high air transport costs.

The need to maintain varieties will be a requirement in perpetuity. Continuous selection after the release of varieties is imposed by the need to eliminate deleterious mutations and exploit any positive source of existing and newly derived variation (Fasoulas, 1993). The genetic variability in a crop always greatly exceeds what one breeder can effectively handle, and this may be observed even in his own early products (Simmonds, 1979). Rasmusson and Phillips (1997) emphasized that elite gene pools have inherent mechanisms to provide a continuing source of new genetic variability. They reported that selection gain occurs due to variation present in the original gene pool as well as due to de novo generated variation such as gene amplification and transposable elements. Nonstop selection is important for exploiting newly derived variation, eliminating deleterious mutations, and securing breeder's seed of optimal quality in every generation.

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Asparagus

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1 Introduction

Asparagus has been a valued vegetable since its early domestication, not only for human consumption, but also for its medicinal properties. Nowadays its cultivation is spread in all continents with steady increments of the planted area due to an increased consumer demand for fresh, canned and frozen asparagus (see Table 1). The largest increases in asparagus production in the last ten years have occurred in countries like Peru and China, which have low labour rates and/or the possibility of marketing their production when prices are high in other countries or hemisphere (Benson, 2002a).

Table 1. Estimated world production area in hectares by continent and year. In parenthesis is expressed the percentage destined to white asparagus in each continent and year.

Continent	Year		
	1989[a]	1997[b]	2002[c]
Europe	52,500 (73)	62,490 (86)	60,575 (82)
Asia	16,970 (29)	68,060 (70)	101,628 (64)
Australian area	6,510 (0)	7,010 (0)	6,687 (0)
North America	45,500 (4)	45,570 (1)	50,810 (1)
South America	11,700 (49)	30,100 (50)	28,800 (42)
Africa	3,250 (92)	5,105 (73)	3,945 (68)
Total	136,430 (39)	218,335 (55)	253,445 (52)

[a]source Nichols (1990).
[b]source Benson (1999).
[c]source Benson (2002a).

Some seed companies have taken advantage of this expansion, with the need and development of hybrids adapted to newly production areas and cultivation strategies; however, the development of new varieties highly productive, tolerant to the most frequent diseases and high market quality will be the challenge of breeders in the coming years.

Many symposia, thesis and research papers have came out since Ellison (1986), last chapter devoted to asparagus breeding in a vegetable breeding book, 20 years ago. Our wish is to reinforce some of the previously stated concepts and review the best strategies to secure highly valuable outputs from a breeding program.

2 Origin and Domestication

2.1 Cultivated Species and Domestication

Asparagus is a large genus comprising about 150 species of herbaceous perennials, tender woody shrubs and vines. Some of them are grown for their ornamental value and foliage, and one of them (*A. officinalis* L.) for food (Bailey, 1942). Three subgenera, *Asparagus*, *Protoasparagus* and *Myrsiphyllum* can be recognized according to Clifford and Conran (1987). The species of the first subgenus are dioecious, while those of the other subgenera are hermaphrodite. *Asparagus* species are naturally distributed along Asia, Africa and Europe. Many of them have economic value as ornamentals (i.e. *Asparagus plumosus*, *A. densiflorus*, *A. virgatus*), or for their medicinal properties (i.e. *A. racemosus*, *A. verticillatus*, *A. adscendens*) (Štajner et al., 2002). Even when the search for young tender shoots as a tasty vegetable of the wild species *A. acutifolius* has cultural roots in Spain and Greece (Ellison, 1986; González-Castañón, per. comm.), the only worldwide cultivated species for tender shoots either blanched (white) or light exposed (green) is *A. officinalis* (hereafter referred as asparagus or cultivated asparagus).

The postulated region of origin of asparagus comprises Eastern Europe, Caucasus, and Siberia (Sturtevant, 1890), where supposedly was domesticated. Greeks and then Romans took the culture of growing asparagus from eastern nations, from which they also took the old-Iranian word ‘sparega’, which means shoot, rod, spray; becoming ‘asparagos’ and ‘asparagus’ in Greek and Latin respectively. One of the first detailed guides on how to raise asparagus is traced back to about 65 A.D. by the Roman Columella (Lužný, 1979). Romans spread the culture of growing asparagus along with their empire throughout Europe. There is also evidence that crusading troops brought asparagus seeds from Arabian countries to the Rhine valley around 1212 (Reuther, 1984). In all Europe, except Spain (Knaflewski, 1996), the decline of the Roman Empire brought a decline in its cultivation, which was confined only to some feudal lords and monastery gardens as a medicinal plant, until the Renaissance, when it was rediscovered as an appreciated vegetable (Lužný, 1979).

2.2 General Botany

The perennial part of the plant is the rhizome (crown), which is composed of clusters of buds with primary fleshy (storage) and secondary fibrous (absorbent) attached roots. The buds sprout rendering the edible organ, a tender growing shoot (spear) between 18-25 cm long, either blanched or not. Once harvests are discontinued, shoots continue to grow becoming the aerial part of the plant (fern), which is responsible for the replenishment of carbohydrates and accumulation until the next harvest season is conducted. The full expanded stems, between a few to 50 or more per plant depending on age, sex and cultivar, have long internodes and can vary in height between 30 and 200 cm. Each stem contains primary and secondary branches where flexuous cladodes (10-25 mm) are disposed in whorls.

In normal temperate field conditions flowering starts at the second year from seed germination, but some plants can flower at the end of the first year. Flowering is in flushes, with up to three flushes per year. Anthesis in each stem begins once it is almost expanded, following an apical direction in the main stem and in primary and secondary branches. Depending on air temperatures, blossom in each stem can last up to two weeks.

Normal sex ratio in out-bred populations and cultivars is 1:1 staminate (male) to pistillate (female) plants; however some hybrid cultivars are composed of entirely male plants. Yellow-reddish male flowers (5-6 mm) and yellow-greenish female flowers (*c*. 4 mm) are disposed in bundles of two or three flower per node, rarely mixed with cladodes (Valdes, 1980). Natural pollination is conducted by bees and bumblebees, and normally male plants flower earlier and produce many more flowers than females. Fruits, reddish berries when mature, bear up to ten (normally 6-8) round black seeds. A 3-year-old or older female plant produces more than 2000 flowers and has the potential to produce more than 10.000 seeds (Machon et al., 1995).

2.3 Dioecy in Asparagus

It is important for the plant breeders to consider the dioecism from an evolutionary point of view. It will help to understand the phenomenon of the different genders and proportions observed in populations and progenies, and to what extent genders are associated to morphological attributes.

It is considered that asparagus has evolved from a primitive hermaphrodite form, via an intermediate gynodioecy state, to the actual dioecious or subdioecious populations, depending if andromonoecious plants (those bearing male and bisexual flowers), are observed (Galli, et al., 1993). The theory tells us that the suggested pathway (Charlesworth and Charlesworth, 1978) involves first a mutation for male sterility, in the case of asparagus this gene being recessive (*X*). This mutation will spread either if the female and hermaphrodite have the same fertility, but some rate of selfing and inbreeding depression occur in the cosexual form, or if no selfing and inbreeding depression are present but some gain in fertility (ovule production) is gained in the female by allocation of resources in comparison to the hermaphrodite state (Carlesworth and Charlesworth, 1978; Charlesworth, 1999). In asparagus,

bisexual flowers are mostly self-pollinated (Thévenin, 1967, Galli et al., 1993); and some inbreeding depression was shown after continued selfing of andromonoecious plants in comparison to unrelated outbred-cultivars (Ito and Currence, 1965) and in the comparison of a Hybrid F_1 cultivar UC 157 (cross of two heterozygous selected plants) and the so-called F_2 progeny (first generation of full-sibs, F = 0.25) (Farías et al., 2004). Regarding to the fertility of female vs. hermaphrodite plants, no strict evidence is available for asparagus, however among females some studies showed association between fitness and morphological attributes; that is, significant positive correlations were found between spear size and seed production (Currance and Richardson, 1937); between stalk height and diameter, and total fruit weight (López Anido, 1996); and between stem height and diameter, positively, and stem number, negatively, with number of berries and seeds per berry (Machon et al.,1995).

Once the gynodioecious state (females and hermaphrodites) has been reached, a second mutation for female-sterility is necessary to reach full dioecy. This mutation, often called modifier, will spread as long as it confers increased pollen production in comparison to the cosexual form. Again there is no clear evidence in asparagus referring the gained pollen output of males vs. cosexuals, however it has been noticed that male flowers are longer than cosexual flowers (Lazarte and Palser, 1979), and this feature has been associated in general to an increased male fitness (Lloyd and Webb, 1977). The modifier can arise tightly linked to the previous male sterility loci or to some extent of independence when it behaves hypostatically, that is not affecting females.

Franken, (1970) studied several selfed progenies of andromonoecious plants and concluded that a partial dominant gene (modifier, *A* in his nomenclature) was responsible for the suppression of pistil development (see Table 2); and this gene was inherited independently of the *X* male sterility locus, previously defined by Rick and Hanna (1943) as a simple Mendelian factor mode of gender inheritance.

Table 2. Phenotypes (genders) and genotypes proposed by Franken (1970) as a model of inheritance of sex.

Pistillate	Staminate	Andromonoecious
XX AA	*XY AA*	*XY Aa* weakly
XX Aa	*YY AA*	*XY aa* strongly
XX aa	*YY Aa*	*YY aa*

Franken (1970) proposed model is also in concordance with the results of Galli et al. (1993) who, after analyzing the length of pistils in some backcrosses, concluded that the factors affecting style length and stigma development (modifiers) are not localized on the chromosome possessing the *X* locus; moreover, the backcross distribution of style length fitted a model of at least two loci.

After a computer simulation, the model of an independent modifier gene for female sterility (*A*) (with no effect on females) arose in a gynodioecious population would spread to fixation, giving a population consisting of females together with either males or modified hermaphrodites depending on the phenotypic effect of the gene (Charlesworth, 1999).

In asparagus, when found, the percentage of andromonoecious plants is extremely low, ranging from 0.1 % (Thévenin, 1967) to 1 % (Sneep, 1953). This is suggesting a high frequency of the modifier gene (*A*) as observed by Franken (1970), and turning the species as if it were segregating for only one gene affecting sex as it was proposed in the earliest model of inheritance (Rick and Hanna, 1943).

The search of the so called supermales bearing *YY*, in the progeny of selfed andromonoecious (*XY*) plants was postulated by Rick and Hanna (1943) in a way to obtain a progeny constituted of entirely males plants. However due to the more complex inheritance of gender in asparagus, as we have seen in the previous paragraphs, extensive progeny tests are required to avoid the presence of andromonoecious plants in the progeny of supermales; that is the super males have to be *YYAA* in order to obtain a strict 100% male progeny (Sneep, 1953).

2.4 Secondary Sex Characters

Secondary sex characters were defined as all differences between males and females in structures other than the sex organs (Lloyd and Webb, 1977). In contrast to what is common in many animals, where sexes can be recognized easily by the secondary sex characters, plants are never so different that their sex could be reliably identified solely by their secondary sex characters in the absence of primary sex structure. In dioecious species these differences (secondary sex characters) might have arisen as a direct or pleiotropic effect of the genes responsible of either male or female sterility (discussed in section 2.3.); or once the dioecy state was reached, intra-gender selection might have taken place favouring the gender output in each sex (indirect effect). In this case some kind of linking to the sex loci are necessary, turning difficult to distinguish between direct or indirect causal effects (Richards, 1990). Machon et al. (1995) studied the sexual dimorphism related to male and female function in asparagus and found that pollen production per flower was positively associated with stem number and negatively with stem height. They also found negative correlations between stem number and characters related to female function (see previous section), and proposed that this morphological constraint could have played an important role in the evolution of dioecy.

In asparagus, since the earliest documented research papers, differences in the mean expression of certain characteristics were associated with gender. It is generally considered that males produce more shoots than females; and females render heavier individual shoots than males (Robbins and Jones, 1925; Lloyd and Webb, 1979). It is important for the asparagus breeder to keep in mind this because, firstly, the economical reason of growing asparagus is the production of shoots, which are not strictly sex structures, but are influenced by the gender of the plant, and secondly, this influence is never so marked to enable a clear separation of genders when observing individual plants. That is, in an asparagus population we are going to observe a great overlapping variation for secondary sex traits between genders as it was shown by Ellison et al. (1960) and Moon (1976).

The quantitative genetic theory dictates that quantitative characters, those presenting continue variation in populations, are considered to be inherited by a certain number of genetic factors, distributed randomly in the genome, with a more

or less important individual effect on the whole character, influenced to some extent by micro and macro-environmental conditions. Asparagus yield, considered either as total spear production in grams, total number of spear per plant or mean diameter of the spear (highly associated to individual spear weight) should be thought as a quantitative inheritance character since, when individual plant data were recorded in populations, a great variation was always reported (Ellison et al., 1960; Legg et al., 1968; Bannerot et al., 1969; Falloon and Nikoloff, 1986; López Anido et al., 1997 among others).

Considering what stated in the previous paragraphs on the direct or pleiotropic effect of sex genes on secondary sex traits, fixation of alleles contributing to sexual dimorphism in linked loci selected for after dioecism was established, overlapping variation in each gender for secondary sex traits and assumed quantitative inheritance; it is reasonable to believe that a great variation for yield, amenable to selection, is still segregating independently from the gender expression. Breeders should consider this to maximize the output of breeding programs, since the success will rely on how much of the total variation is utilized.

2.5 Niche Differentiation and Sex Ratios

As we discussed previously, inter-gender competition for reproductive output could be an essential requisite in the evolution of dioecy (direct effect), and once dioecism has been reached, within and between-sex competition can further operate in a way to maximize the reproductive success. As a result of the gender differentiation, reproductive success in males, pollen production, is considered to represent much less cost than female output, which not only involves flowering, but bearing and nourishing fruits the time required for seed development and dispersal.

In this context provided the same optimal homogeneous growing conditions, one gender (often males) could be favoured and explains the general excess to the expected 1:1 sex ratios of males in many dioecious species (Lloyd, 1974). However when conditions are neither optimal nor homogeneous, some kind of niche differentiation could evolve as long as one gender has optimal reproductive output than the other in one specific niche and vice versa. Due to the distance of pollen travel, the niche differentiation may be limited to small-scale patchy areas in non-wind pollinated species (Richards, 1990).

The niche environment could be characterized by different soil, light or biological factors (i.e. wet vs. dry, illuminated vs. shaded micro-areas, pest tolerance vs. pest susceptibility); and would render different sex ratios in each particular niche.

When assessing sex ratio (sexing) in perennials it is important to distinguish between sex ratio of genets (each genotype grown from a seed in a population) from sex ratio of ramets. Due to the perennial nature many growing points of a genet could separate from each other and constitute independent plants, which in reality are ramets or clones from an original genotype (Richards, 1990).

In asparagus there is no clear evidence of niche differentiation, however some type of gender differentiation consisting of more whorls per unit branch in male plants and greater leaf area per cladophyll and per 10 cm of branch in females found

by Benson (1982) could be traduced in specific optimal light intensity efficiency use by each sex.

In relation to sex ratio influenced by survival, there are some controversial results in the literature. Tiedjens (1924) clearly concluded that in a six years old production field, ´staminate plants die out sooner than pistillate plants´. He also stated than after five year disintegration takes places where old stems of a crown (original genet) have been removed, originating separate clusters of shoots emerging from more or less separate parts of the original crown, and instead of one plant we may find two or three (ramets). The underlying point on his observation, is that unless a careful area delimitation occupied by each genet in the original planting or sowing, that is wider inter crown plantation, or identification on each ramet belonging to each genet as long as they separate; the sexing of old asparagus fields would renders sex ratios much more representative of ramet sex ratios than genet sex ratios. Considering the nature of males to produce more shoots, the ramets sex ratios would be in an excess of males. These may explain the high sex ratios found by Yeager and Scott (1938) 2.5 and 1.4 males : 1 female when sexing a 35 and 14-year-old plantings respectively.

In contrast to Tiedjens (1924) results, Bannerot et al. (1969) evaluating individual plant data in some nine-year-old populations, found a general greater mortality for females (17%) than males (7%), however, the differences in survival plants were more marked in some populations than in others. Also Bouwkamp and McCully (1972) found an increased mortality in females along years, and competition from males failed to account for this higher mortality; what would have been expected to occur if field conditions favoured, in any way, niche differentiation for males.

3 Varietal Groups

3.1 Origin and Ploidy Level

As we have seen in section 2.1, the Renaissance brought an increased interest in neglected species, becoming the growing of asparagus popular in the sixteenth and seventeenth centuries in Germany, France, England and The Netherlands. Asparagus populations began to be identified according to countries and towns where they were grown, arising proveniences as Riga, Ivancice (Lužný, 1979), Ghent, Ulm, Vendome, Besancon (Knaflewski, 1996), Violet Dutch (Kidner, 1947). This latter population is recognized to be one of the oldest, and it is believed that constituted the source from which many subsequent proveniences and thus modern cultivars were developed (Kidner, 1947; Knaflewski, 1996). During the nineteenth century some proveniences as Argenteuil from France and Braunschweiger from Germany gained reputation, replacing old populations and landraces currently planted by that time. Subsequent selections were conducted from Argenteuil in many countries yielding Early Argenteuil and Late Argenteuil in France, Reading Giant in England and Palmetto in the USA. From Braunschweiger, cultivars as Ruhm von Braunschweig and later Schwetzinger Meisterschuss, Huchel´s Leistungsauslese and Lucullus hybrids were developed in Germany (Knaflewski, 1996, Greiner, 1990) (see Fig 1).

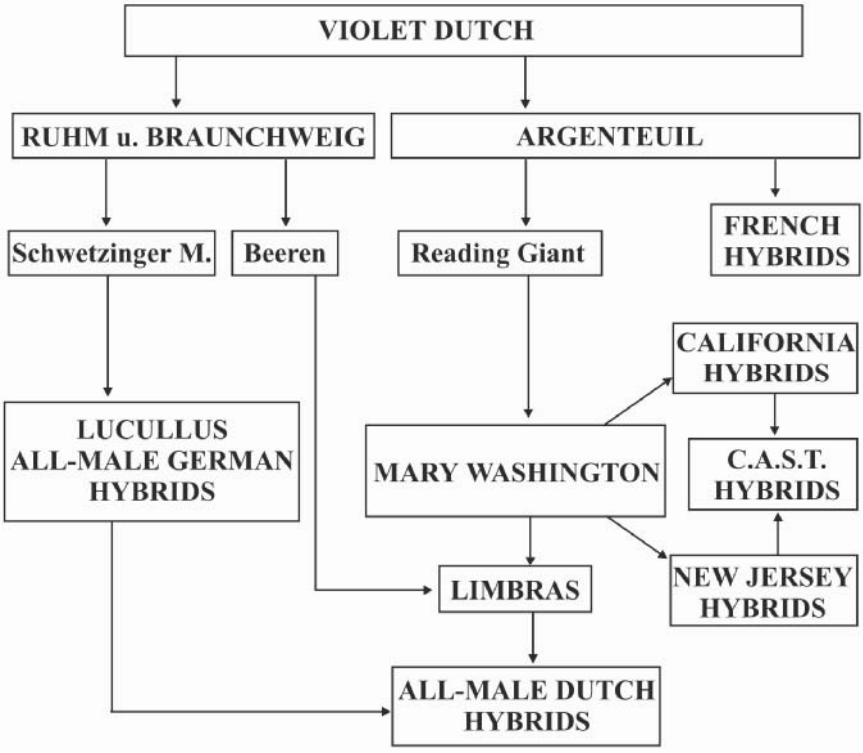

Fig. 1. Genealogy of major asparagus cultivars, adapted from Knaflewsky (1996).

In the USA, in the beginning of the twentieth century, a great effort of breeding was carried by J.B. Norton in the search for rust (*Puccinia asparagi*) resistance, yielding Martha Washington and then Mary Washington cultivars. Each cultivar was the advanced first generation progeny of two selected plants derived from Reading Giant and probably Argenteuil (Ellison, 1986; Knaflewski, 1996). In California, subsequent selections on Mary Washington rendered cultivars as UC 500 and UC 309 (Hanna, 1952), and then further selections on UC 500 gave rise to UC 157 hybrid (Benson and Takatori, 1978). In New Jersey, selections were also based in Mary Washington, yielding some male hybrids as Greenwich, among others (Ellison and Kinelski, 1986). In Taiwan improved materials were derived from UC 157.

Both in France and Italy, selections of advanced cultivars were derived from Argenteuil; however, in Italy, selections from foreign materials were also included in a latter scheme (Falavigna et al., 1999).

In The Netherlands formal breeding programs started in the mid twentieth century from Ruhm von Braunschweig. Inbred (full-sib) selection was conducted, rendering a great number of inbreds, which in turn gave rise first to Limbras and then Boonlim, Backlim and Thielim hybrids. In the last period, a foreign material, UC

157, was included as germplasm to improve earliness (Van den Broek and Boonen, 1990; Scholten and Boonen, 1996).

In this context, from a varietal group point of view, even when it is believed that almost all present cultivars are derived in any way from Violet Dutch, cultivars from USA, Taiwan, France and Italy could be gathered as derived mostly from Argenteuil and those from Germany and The Netherlands derived from Braunschweiger. This is in concordance with the results of Geoffriau et al. (1992), who found that The Netherlands and Germany accesions tended to group together well differentiated from materials of USA, Italy, Spain, Taiwan and France when evaluated for the variation present in a seven enzyme systems.

All these described cultivated forms so far are diploids (2n = 20). There are some tetraploid local landraces as Morado de Huetor in Spain (Moreno et al., 2006), Violetto d'Albenga in Italy (Falavigna and Fantino, 1985), Cereseto and Poire in Argentina (López Anido et al., 2000), and derived cultivars as Purple Passion (Benson et al., 1996) and Purple Pacific (Fallon and Andersen, 1999) which could be considered as a distinct (tetraploid) varietal group. Their spears are recognized by showing intense anthocyanin pigmentation when grown exposed to sunlight, and, as suggested by Kay et al. (2001), they are not simple autotetraploid derivatives from diploids *A. officinalis*, but probably derived wholly or partially from tetrapoids *A. maritimus* or *A. prostratus*.

There are also some tetraploid cultivars derived artificially from cultivated diploid forms (Skiebe et al., 1991, among others), but they should be considered a separate tetraploid varietal group from the origin point of view.

3.2 Production Type

A given asparagus material could be cultivated either for blanched or green spear production. However, in general, breeding programs in each country have been carried out considering the primary form of consumption. At that respect, rendering that American cultivars are in general more suitable for green spears, meanwhile German, French and Dutch materials are more appropriated for white spears production.

4 Breeding Methods and Techniques

4.1 Per se vs. Progeny Values

Before giving details of the different methods conducted in the almost one hundred years of documented breeding in asparagus, we will recall what is of primary importance when dealing with quantitative characters; that is, the differences between judging the merit of a genotype upon its individual *per se* performance (used in mass selection) or upon its progeny outcome (used in family selection).

In the case of a perennial as asparagus, the microenvironment surrounding the plant plays a crucial importance because its effect (positive or negative depending the season) will shape the phenotype incrementally or not along seasons. Also due to

mortality, some plants will be less affected by neighbours, showing a phenotype more associated to lack of competition than to the merit that the genotype would express under an even stand density. All these explain in part the highly variation in the rankings of individual plant yield along years as pointed by Hanna (1938) and the relative low values of broad sense heritability for yield traits expressed in a single repetition and environment basis found by Legg et al. (1968) and López Anido et al. (1997).

Currence and Richardson (1938) first pointed out the importance of progeny tests as a measure of the yielding ability in asparagus, and observed a closer relationship between male parent and progeny spear size than between male parent and progeny annual yield. Later, Currence (1947) extended the studies to bi-parental crosses, and found a correlation coefficient of 0.259 between mean yield of the two parents and their progeny output, concluding that due to this low correlation no special emphasis should be given in selecting vigorously parents in a way to attempt greater yielding progenies. The same correlation (calculated for spear diameter upon data of his report) was of 0.688; that is, the parental values would be more predictive of the value of a progeny for spear size than for total yield. Also, as we are going to discuss later, the low correlation found between parental *per se* and progeny values for yield could be indicative of genetic actions involved other than merely additive.

Huyskes (1959) found that a male plant only pollinates those females which grow very close to it, thus, the value of open pollinated seeds (half-sib progenies) should be taken with caution and bi-parental or bulk pollen assisted crosses should be preferred.

4.2 Additive vs. Non-additive Gene Actions

Even when asparagus hybrid varieties has been long pursued in breeding programs, reports dealing with the major gene actions and variances involved in the expression of yield traits are scarce. Ito and Currence (1965) found significant high-parent heterosis among inbreds, however no cross was significantly more productive than the commercial cultivars included as checks. Rameau (1990), found high-parent heterosis of more than 100% in three of the four crosses evaluated. The highest cross yielded around 3 T ha^{-1} and no data on check cultivars were included.

Unfortunately, in the literature, there are few reports that presented data on yield of a given set of crosses. Among those, Currence (1947) and Price and Baughan (1990) included complete yield data on a set of males crosses to a set of females (circular partial diallel scheme), from which general and specific combining abilities could be inferred. After applying the Viana (1995) model of analysis to Currence (1947) and Price and Baughan (1990) data, we found that hybrids yield was explained (R^2) in a 56 and 64% by the general combining ability (g_i) of the parents; and the relative importance of the general combining ability variance upon the variance due to crosses was of 0.57 and 0.65 respectively. Similarly, Asprelli et al. (2005) found that the Baker (1978) coefficient, which expresses the relative importance of the general combining ability variance upon the total genetic variance, was of intermediate nature for total and marketable yield. All these reports so far are

indicating that general ability and specific ability effects are of equivalent importance in determining the yield output of hybrids.

4.3 Heritability, Recurrent Selection and Heterotic Patterns

As we have seen in the previous section, the performance of hybrid progenies in asparagus is expected to be equally determined by the general combining ability of the parents (related to additive gene action) and the specific combining ability of the combination (related to non-additive gene actions). In this context, in order to secure the highest hybrid performance, breeding programs should devote efforts in maximizing both types of gene actions. The frequency of alleles contributing additively is improved by means of recurrent selections schemes. López Anido et al. (1999) and Gatti et al. (2005), evaluating full-sib and half-sib Argenteuil derived families respectively, found relative high values of strict sense heritability for marketable yield, marketable spear number and spear diameter on a family mean basis. The latter authors also estimated a 3.2% of annual marketable yield increase by applying the half-sib selection scheme, with five years required to complete each cycle. It is of interest to note the reciprocal recurrent selection scheme presented by Rameau (1990), in which one population is improved for spear diameter and the other for spear number. No empirical data on the effectiveness of this scheme was presented, but taking in account the importance of additive gene actions on the performance of progenies, and that the highest marketable yields are always attained by good number of thick spears, her scheme sounds more than reasonable.

In a way to maximize the potential heterotic effect (related to non-additive gene actions and epistasis), parentals drawn for hybrid crosses should be secured from populations as divergent as possible. However, as we have seen, cultivated diploids forms of asparagus were derived from a relative recent common origin. There is no evidence of any clear heterotic pattern for yield, but some breeding programs intuitively included parents with some kind of divergence from the early beginning (Benson et al., 1996), and others expanded the array of parents to include some from different origin (Van den Broek and Boonen, 1990; Falavigna et al., 1999; Roose et al., 2005).

4.4 Performance vs. Uniformity

The ideal asparagus cultivar which any breeder envisages is one highly productive, uniform, all-male (but not necessarily), as tolerant as possible (or resistant) to diseases and adapted to a target type of production and macro-environment. The different breeding strategies we will discuss below, in general, emphasized pursuing either high performance or uniformity and in this way some discrepancies are observed when comparing their outcome.

4.4.1 Mass Selection

This is the simplest and oldest method. It involves the identification of superior plants and the pollination of those selected to conform the improved generation. Both

males and females parents should be selected to attain the maximum response. This needs transplanting elite plants to an isolated field or elimination of all not selected flowering male stalks from the original selection plot. Its success will rely in to what extend the phenotypes selected are the expression of additive gene actions or merely the result of positive micro-environment deviations as previously discussed. By means of only leaving selected female plants for seed bearing Adam and Skiebe (1964) obtained an 18% increment in yield, and proposed this method to, at least, maintain population attributes along years. Chen et al. (1987) applied mass selection in the development of varieties Tainan Nº 1, 2 and 3 with an average 10% increase in total yield over original populations under the mother stalk harvest system. No precise information was given on how many cycles of selection were applied, supposedly just one.

In view of the relative high responses attained, the implementation of recurrent mass selection schemes in a way to maintain and gradually improve populations to secure sources for elite genotype derivation should be considered in breeding asparagus.

4.4.2 Family Selection

The identification of superior genotypes (those with accumulated favourable alleles contributing additively to the character of interest) would be more effective under progeny tests as discussed previously. That was the method applied by J.B. Norton to select the parents of varieties Martha Washington and Mary Washington with an increment of up to 17% in mean spear weight over the traditional Palmetto cultivar (Ellison, 1986). The new populations were conformed by the advanced open pollination of the selected parents. One interesting point to notice is that Norton's cultivars and subsequent selections (conducted mainly from Mary Washington), are essentially derived from two selected plants. Thus a kind of bottleneck could be operating restraining the potential allele accumulation to those included in the original pair of plants. Adam and Skiebe (1964), by means of progeny evaluation, proposed the conformation of advanced polycrosses of the progenies that expressed best combining ability.

4.4.3 Inbred Lines and Hybrids

Different types of hybrids and techniques to obtain their progenitors were proposed and used in asparagus.

4.4.3a. Selfing. The use of andromonoecious or hermaphrodite plants in a way to advance inbreeding in a shorter time than full-sibbing was proposed by Sneep (1953) and Marks (1979). It is a well known principle among breeders that the potentiality (favourable alleles) of an inbred is determined by the S_0 or F_2 plant (in the case of autogamous crops) from which it was derived. Thus, it is recommended to secure as many different S_0 derived lines as possible to increase the probability of obtaining superior lines. In this context, and due to the general extremely low frequency of

bi-sexual bearing plants (S_0) in asparagus, it is doubtful that by this method an elite line with many fixed favourable alleles could be secured.

4.4.3b. Full-sib inbreeding. This method for advance inbreeding is not as rapid as selfing. The number of cycles would depend on the uniformity required by the breeder, but not before seven generations are obtained that the F value will reach 0.73.

However, it provides both a greater opportunity to select among inbreds (there is no limit in the number of different sib-mating lines that can be derived from a population) and a greater opportunity to select within each line (major proportion of the additive variation in a plant basis is expressed within). This method was used to develop a large array of advanced inbred lines in The Netherlands (Scholten and Boonen, 1996), where at least six generation were required to attain a reasonable degree of homozygosis. By the use of a heated greenhouse, each generation could be attained in two years. After evaluating the best combinations, Limbras dioecious hybrids were released first and all-male Boonlim, Backlim, Thielim and Horlin later (Scholten and Boonen, 1996). These materials, in general, have shown an excellent performance and have widespread use in The Netherlands. As large amount of seeds are available during the inbreeding, appropriate field evaluations can be conducted, as well as coupling diseases scoring and selections. This was the method used in developing the botrytis resistant Lucullus hybrids in Germany (Ellison, 1986).

4.4.3c. Polyembryony seeds bearing monoploids. This phenomenon, which is expressed as two or more seedlings emerging from a single seed (multiple seedling), was studied and proposed in asparagus as a quick method of obtaining pure lines after doubling chromosome number of monoploids (Randall and Rick, 1945). The frequency of multiple seedlings found by these authors was 0.95%, and from those the proportion of monoploid plants was 0.016 (the greatest proportion of multiple seedlings corresponded to diploids). Thus the final number of monoploids identified was of 1.5 every 10 000 evaluated seeds.

Later Bassett et al. (1971) proposed the use of populations fixed for the recessive allele responsible of anthocyanin red-stalk colour as a way to make the detection of monoploids more efficient. It is expected that, in these populations, after pollination with dominant green-stem colour males, all seedlings possessing the visible red-stalk marker would be of monoploid nature from parthenogenesis. From 19,292 seedlings, 10 were coloured and from these nine monoploids (90% efficiency). The final proportion of monoploids found by these authors was 4.6 every 10 000 seeds, quite similar to previous authors data. All monoploids are female, thus, genotypes should be maintained and evaluated by vegetative means. To overcome this problem Thévenin and Doré (1976) proposed the incorporation of the male locus to homozygous female plants and, by means of backcrossing (5 to 6 generations), obtaining seed propagated inbreds with the normal sex ratio, near-homozygous and near-isogenic between genders.

The same concepts discussed with the selfing method applies in this case; that is, the extremely low frequency of recovered inbred out from a population narrows the number of genotypes that could be fixed to a relative low number, limiting the

potential advance that can be secured. This method was used extensively in the French breeding program. The mother line of the first released true F_1 all-male hybrid (Andreas) (Corriols et al. 1990) and the 58% of the homozygous lines evaluated in crosses by Denis and Rameau (1994) were derived from polyembryony seeds.

4.4.3d. Double hybrids. They were proposed as a way to maximize the heterotic response found in crosses of selected heterozygous plants. In contrast to the classical maize's coined concept of double hybrids, built by the cross of four inbreds, in asparagus, they are formed by the cross of four selected heterozygous plants (Corriols-Thévenin, 1979). Both the males in the parental female F_1 and the females in the parental male F_1 are eliminated for the hybrid seed production. To obtain the best combination, information on single hybrids between heterozygous selected plants is required. Yield predictions of all possible double hybrids can be advanced as proposed for maize (Hallauer and Miranda Fo, 1988). In the French breeding program, from 1974 to 1977, four doubled hybrids (Diane, Junon, Minerve and Larac) were released with an average 30 and 60% increase in total and early yield over standard cultivars (Corriols-Thévenin, 1979).

4.4.3e. Clonal hybrids. The possibility of obtaining a great number of cloned plants out of a selected asparagus genotype by means of tissue culture (Murashige et al., 1972), redirected breeding programs in a way to capitalize the hybrid vigour expressed in the single cross of two selected cloned heterozygous plants (cloned hybrid). UC 157 (Benson and Takatori, 1978) and Ida-Lea clonal hybrids were released, acquiring the former great popularity and widespread use. Later Benson et al. (1996) presented Apollo, Atlas and Grande, with significant higher yields over UC 157. Benson called Apollo a clonal 3-way hybrid because the male parent was selected out from a clonal hybrid and the female from a population. Atlas and Grande were defined as di-clonal hybrids because both parents were selected from a clonal hybrid (Benson per. comm.).

In a way to avoid confusions, we do not believe that any appreciable differences either in potential hybrid vigour or uniformity would be expected to justify the use of new terms other than clonal hybrid in these cases. In the French breeding program five clonal hybrids were released (Aneto, Desto, Cito, Bruneto and Steline) with an average 75% yield increase over a commercial check (Corriols-Thévenin 1979).

4.4.3f. Anther culture – doubled-haploids. In vitro-anther culture in asparagus has proven to be an efficient method to obtain haploids via androgenesis (Doré, 1974). After 4 to 6 weeks on a suitable medium androgenetic calli or embryoids are developed from one or a few microspores. These calli are not haploid but mixoploid. When transferred onto a regeneration medium a callus or an embryoid grows and then pre-organized structures initiate adventitious shoots and stems of one ploidy level. Repeated cultures and microcuttings of these stems are necessary to obtain entire plantlets (Doré, 1990).

Either by spontaneous chromosome doubling at the callus stage or later induced by colchicine, this method renders homozygous supermales or females. This

technique has been long used by the Italian breeding programs (Falavigna et al., 1999), where up to 1400 doubled haploids were developed and screened. Selection of vigorously anther donor plants was conducted first in old fields of the adapted Argenteuil cultivar and later over foreign materials as well. Male doubled-haploids are subjected to progeny test to distinguish supermales (*YY*) from those with a somatic cell origin (*XY*). Screenings for at least three years in *Fusarium* infected soil, including rust tolerance ratings and plant vigour are conducted to select the double-haploid clones, progenitors of the experimental hybrids to be further evaluated. During the 1981-1993 period 280 experimental hybrids were evaluated.

Two distinct types of hybrids were developed, two-way all-male hybrid, which is the classical F_1 from two homozygous doubled-haploid plants, and three-way all-male hybrid, which is the cross of a heterozygous female plant and a double-haploid supermale. Two-way hybrids Ringo and Golia and three-way Argo and Eros were released with an increased yield from 50 up to 100% over open pollinated varieties (Falavigna et al*.,* 1996). Also, two-way hybrids expressed greater uniformity than open pollinated varieties, being three-way materials intermediate.

From the breeding point of view, among the methods available to reduce the time required to attain homozygosis, anther-culture offers a greater advantage, since many more different homozygous genotypes could be secured, enhancing the opportunity for selection. Also, in contrast to what is necessary in the case of supermales derived from andromonoecious plants (see section 2.3), no progeny evaluations to avoid the expression of bi-sexual flowers are required. However some problems were also mentioned as insufficient *in vitro* embryo yield and low percentage of plant regeneration for certain genotypes, possible regeneration of heterozygous males (somatic origin), difficulties to distinguish *in vitro* diploid from polyploid genotypes on morphological traits alone, up to 41% of regenerated clones lost because not been diploid, long time needed to evaluate the clones in the field and difficulties on micropropagation of some doubled-haploids parents of selected hybrids (Falavigna et al. 1999).

4.4.4 Clones

This proposed breeding strategy directly makes use of the feasibility to sufficiently micropropagate asparagus selected genotypes (clones) in order to establish production beds (Nikoloff, 1988). Its success will depend, as we have seen in section 4.1., on the skill to identify a superior phenotypic performance on a plant basis and to what proportion this is attributable to a reproducible genotype.

Prior decision making on clone recommendations, evaluations in replicated trials are necessary, since some cloned genotypes may be as variable in plots as populations raised from seeds. When expressing yield output in a hectare basis, mean yield of some clones proved to be 2 or 3 times grater than populations from where they were extracted. However, when comparing yield at a similar chronological age and in an individual plant basis, performance of clones are lower than the selected parent plants (Nikoloff, 1988). This was related to some carry over effects from micropropagation media. Probably the ancymidol, used to encourage *in vitro* storage root formation, caused that clones partitioned more of their dry matter to roots and

less to shoots than did the conventional seedlings from seeds as suggested by Fisher et al. (1996).

Due to the lower influence of microenvironment on quality attributes as spear diameter, tightly closed heads and anthocyanin pigmentation, it is strongly recommended to give more emphasis on these characters when selecting elite genotypes for cloning.

Fraser-Kevern et al. (1996) showed in an economic analysis of clones vs. seed grown Jersey Giant, for the New Zealand conditions, that the grower of clones would receive after the fifth year a gross margin of $26,710 per annum in comparison to the $5,300 per annum when using Jersey Giant. These authors speculate that in the short-term tissue culture companies and nurseries will refine their techniques to ensure transplant at a reasonable price and suggested that growers will carefully assess the cost and benefits of clones and, in the longer term, a significant proportion of the crop will come from clonal plantings; thus revolutionizing asparagus breeding as Nikoloff (1988) envisaged.

5. Breeding Achievements

5.1 Correlations

The asparagus peculiarities of being a perennial with harvests starting not before the second or third year after seed germination and prolonged many days each season motivated breeders, from the early beginning, to look for less-time consuming alternatives in judging plants and progenies.

Cultivar evaluation along the first two harvest seasons proved to be a reliable estimator of potential yield of longer periods (Bussell et al. 1987), and in a single plant basis, selection for spear size could be effectively conducted considering solely first year harvest data (Cointry et al., 2000).

In order to reduce data recording on progeny yield along all season, early yield, measured the first couple weeks, has proved to be highly correlated with total season yield (Ellison et al. 1960). More recently, Stone and Roose (2002) proved that Monday-only data recording in a three harvests per week scheme (Monday, Wednesday and Friday) was highly correlated with full season data.

Number and size of sprouted buds along harvest (yield) and once cuttings are interrupted (summer ferns) are the expression of the same genotype attributes as early pointed by Tiedjens (1924). These impelled breeders to search for alternatives to spear harvest data recording by means of summer stalk screening. From all the characters evaluated in the literature, both in an individual plant or plot basis, the highest correlations with marketable yield were found when a kind of fern index was calculated weighing total number of summer fern by stalk diameter (Ellison and Scheer, 1959; Thévenin, 1967; Wolyn, 1993) or by assessing the circumference of the area from which summer stalk emerged (Knaflewski, 1985).

In order to reduce the time comprising each cycle of selection, Van den Broek and Boonen (1990) proposed a method in which, by means of transplanting to field conditions three-month old seedlings and harvesting the following two seasons, the

time to complete a selection cycle will be only two years. The reliability of this method was confirmed later by Scholten and Boonen (1996).

5.2 Tight Heads

Tips composed of tightly closed heads, smooth buds, compressed scales covering lateral buds are of primary importance under green asparagus production (Ellison, 1986) (see Figure 2). In warm climates or hot days in temperate climates considerable differences are attained between selected and unselected cultivars. UC 157 and derived materials in general have shown excellent attributes for these traits, which have been proven to be highly correlated with the height of the first branch of the summer stalks (Ellison, 1986).

Fig. 2. From left to right spears showing increments in head tightness; the first two are not suitable for market, the last is the most desired.

5.3 Light Coloured Heads

White asparagus is preferred in general without a purple spear tip, which is either an indication sign that the spear was exposed to light at the time of harvest, related to an older emerged spear, or that was quite long when it was harvested, associated to

fibrousness accumulation. Genetics of anthocyanin pigmentation has been proposed as a two loci model, with one dominant epistatic allele in one locus (*I*), and a recessive hyphostatic allele in the other locus (*p*) inhibiting colour (Peirce, 1982). In this way any progeny fixed for *I* or *p* will show light coloured asparagus tips. However, in materials exhibiting pigmentation, some degree of variation is observed for the colour and intensity of the scales (gray, brown, purple), being this an indication that some other modifier genes are involved.

Among others, developed cultivars without anthocyanin colouring are Spaganiva, Aarslev 136 and Emerald. In certain countries as Denmark, this attribute is very important for the market acceptance (Sørensen, 1996).

5.4 All-male Hybrids

Since Rick and Hanna (1943) demonstration of all-male attained progenies, several breeding programs have been focused in all-male cultivars as a primary task. The reason was already discussed when dealing with secondary sex traits. However as soon as all-male outputs were evaluated in comparative field trails some discussion arose on whether they are convenient or not (Corriols, 1984). Table 3 summarizes mean ranked data of four reports where different types of dioecious and all-male cultivars were evaluated.

Table 3. Mean marketable yield ranking of different cultivar types from four data sources.

	Mean Marketable Yield Ranking				
	Cultivar type				
	Dioecious				All-male
Source	Population	Double hybrid	Clonal hybrid	Simple hybrid	Clonal and Simple hybrids
Corriols (1984)	29	14	14	13	20
Nikoloff et al. (1986)	15	9	4	7	12
Nikoloff and Falloon (1990)	-	-	6	-	10
Benson (2002)	13	14	9	-	14

It is apparent, as previously stated by Corriols (1984), that the fact of being all-male does not guarantee highest yields. What it assures is the absence of volunteers from dropped seeds. Nikoloff and Falloon (1990) attributed the unexpected poor performance of all-male hybrids to a low selection pressure imposed on supermales. As we seen in section 2.3, due to the low frequency of andromonoecious plants in populations, high selection pressure on supermales would be hardly possible unless supermales are derived from doubled haploids (anther culture). We should also remember that appreciable variation is segregating independently of gender and highest marketable yields are always attained by a good number of thick spears. These concepts should be considered in breeding program pursuing competitive all-male outputs.

6 Current Goals of Breeding

6.1 Genotype by Environment Interaction

Due to the perennial nature and the unpractical cultivar replacement once plantation has been established, the choice of an appropriate material to be grown is of crucial importance for the asparagus grower. A suitable cultivar could be one with wide adaptation or one that interacts favourably with the environment conditions maximizing yields. In this context, the interpretation of the genotype by environment interaction and the possibility to take advantage of any interaction are of crucial value for the breeder's strategy. Contreras and Krarup (2000) found significant cultivar by environment interaction for growth attributes, being some hybrids more sensible to an environment gradient than others, thus taking benefit of favourable conditions. The open cultivar (Mary Washington), even more erratic, was more stable (*b* value) across environments than hybrids.

In France, by means of factorial regression model, genotype by environment interaction has been best explained by covariates as latitude, type of production (white or green) and mean temperature of the five months preceding the beginning of the harvest (Rameau and Denis, 1992). As expected, doubled hybrids (as Larac) showed more adaptability than single hybrids (as Andreas); which was only suitable for the North of France. More recently Nichols et al. (2002) have proposed a two-stage imputation method to study the genotype by environment interaction under incomplete data arrays. First a cluster analysis for similarities in shape under an environment gradient (same shape behaviour) is conducted, and then an assessment of different levels under same shape (best cultivar under same behaviour) is analyzed.

As we have seen in section 1, since the last years the increase of the asparagus planting area has been concentrated in countries where neither asparagus was a traditional crop nor breeding programs were developed. That means that breeders should also consider pursuing outputs with either a wide adaptation or suitable to the specific new planting areas conditions. In general, as common sense dictates, asparagus materials breed in warm climates should be more adapted to any newly warm or tropical planting areas than moderate or cooler climates breed outputs. The opposite would occur for new moderate climate areas.

6.2. Disease Resistance

6.2.1 Fusarium Wilt

Fusarium stem, crown and root rot caused by the soil-borne *Fusarium moniliforme* Sheldon, *F. proliferatum* and *F. oxysporum* Schl. f. sp. *aspargi* Cohen is one of the most prevalent and injurious diseases in asparagus field all over the world, associated to the asparagus field decline along years and in some instances prevent asparagus implantation. Resistance has been reported in *Asparagus densiflorus* cv. *Sprengeri* (Stephens et al., 1989), however, attempts to transfer this resistance to *A. officinalis* have been unsuccessful (Marcellán and Camadro, 1996).

In order to take advantage of the observed genetic variation in resistance to *Fusarium* within *A. officinalis*, attempts as *in vitro* selection assay (Tu et al., 1985; Lassaga et al., 1998) and gametophyte selection (Pontaroli and Camadro, 2001) were proposed.

In general, evaluation of breeding stocks in breeding programs are currently advanced under some *Fusarium* spp. infected soil or nursery to access tolerance under field conditions.

6.2.2 Stemphylium Leaf Spot

Purple spot on spears or leaf spot on cladophylls and stems are caused by *Stemphylium vesicarium* (Wallr.) Simmons and may produce severe defoliation of summer ferns, affecting potential yield. Under controlled environment and inoculation all *A. officinalis* accessions evaluated showed high levels of infection. Resistance has been found in some asparagus species, with *A. asparagoides* (L.) W.F. Wight, *A. compactus* Salter, *A densiflorus* cv. *Sprengeri* and cv. *Myersi*, *A. larcinus* Burch, *A. verticillatus* L. and *A. virgatus* Bak being highly resistant (Bansal et al. 1986). Under field prevailing conditions for natural infection, however, some different levels of tolerance can be observed among cultivated materials. In general French cultivars were more susceptible than North American breeds. This was attributed to distinct plant architectures, being French cultivars shorter, lower branched and more compact than North American's, producing a microenvironment more favourable for the fungus (Broadhurst, 1996).

6.2.3 Phytophthora Rot

Phytophthora species as *P. megasperma*, *P. megasperma* var. *sojae*, *P. crytogea*, *P. cactorum* and *P. richardiae* are responsible for the rot disease, which may cause severe reduction in asparagus stands in the first years after transplantation, affecting also the quality of harvested spears (Falloon, 1985). A nursery inoculation and growing protocol was proposed to assess sources of tolerance and, by means of recurrent phenotypic selection, obtain an improved population for derivation of highly tolerant lines (Falloon, 1990).

6.2.4 Rust

As discussed earlier, the severe rust outbreak caused by *Puccinia aspargi* in New England in the beginning of the last century has promoted the search for sources of resistance and established the bases of modern asparagus breeding. Later rust resistance was not maintained in Mary Washington, because the foundation stock was no longer available and growers used seeds from open pollination to establish new fields (Ellison, 1986).

More recently, Johnson and Peaden (1993) assessed the area under the disease progress curve (AUDPC) in a set of ten crosses from genotypes expressing different levels of resistance. The distribution of the AUDPC was left skewed, indicating some degree of dominance, and the parent-offspring heritability was 0.55, suggesting

that to some extent additive genetic variation was responsible for the resemblance of parents and progenies. In many breeding programs, progenies out from selected genotypes or selected micropropagated genotypes themselves are routinely nursery screened for rust performance under natural occurring inoculum.

7 Genetic Resources

7.1 Opportunity of Use

The concept of genetic resources is extended to all materials plausible of being used for the maintenance and improvement along time of yield, nutritional quality, adaptability, resistance and cultural heritage of cultivated species. It includes breeding stocks, old cultivars, landraces, wild ancestors, related species and weedy forms.

In asparagus as we have seen in section 3.1, the majority of the currently grown cultivars are traced to a common original source population. Nevertheless some attempts to widen the genetic range have been conducted. Ito and Currence (1965) included advanced inbreds derived from the cross of *A. officinalis* x *A. brachyphyllus*. Mc Collum (1988) crossed colchicine tetraploids of *A. officinalis* to *A. prostratus* and advanced two fertile F_2 populations. Ito et al. (2005) studying phylogenetics in a wider array of asparagus species, secured progeny in the cross of *A. officinalis* x *A. kiusianus*, and concluded, as it is generally expected, that the potential of the interspecific hybrids is linking to the phylogenetic distance.

Within the cultivated germplasm, hybridizations between diploid and tetraploid forms have been conducted artificially but with a very low frequency of viable seed recovery (Wagner and Ellison, 1964; Ozaki et al., 2004). This and the minority cytotype exclusion principle (Levin, 1975) may explain the maintenance of some tetraploid landraces in Spain, Italy and Argentina without contamination from the more frequently cultivated diploid forms. Moreno et al. (2006) suggested the potential value of these landraces to widen the genetic diversity of cultivated diploid germplasm. Also wild types as Triguero (González-Castañón and Carbajal-Carcedo, 1996) could be of interest since it showed some divergence from cultivated forms.

7.2 Conservation Status

Compared to other crops the conservation of the genetic resources of vegetables in general and of leafy vegetables (as asparagus) in particular has received relatively little attention (Cross, 1998). Hintum and Boukema (1999) estimated that the number of leafy vegetables entries in world collections (mainly *Lactuca*, *Cichorium* and *Spinacia*) represents less than 0.5 % of the total plant accessions.

In asparagus, actions were suggested to establish an international asparagus germplasm collection (Jermyn and Cross, 1999); however, it had received until now, both in general and in the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) in particular low priority (Lebeda and Boukema, 2001). As in many European countries the general process of genetic erosion in leafy

vegetable landraces and wild relatives is noticeable, these latter authors stressed the urgent necessity to implement collecting expeditions to counteract this process. Table 4 summarizes the major germplasm collections holding *Asparagus* accessions.

Table 4. Major germplasm collections holding accessions of *Asparagus* species by country.

Source	Country	Institution / Responsible person	Species	Number of accesions	Online data base and query
Lebeda and Boukema (2001)	Hungary	Institute of Agrobotany Dr. Aspád Kiss akiss@agrobot.rcat.hu	*officinalis*	2	No
	Poland	Research Institute of Vegetable Crops Dr Teresa Kotlinska tkotlin@inwarz.skiemiewice.pl	*officinalis*	54	No
		National Centre for Plant Genetic Resources Dr. Wieslaw Podyma w.podyma@ihar.edu.pl	*officinalis*	38	No
	Spain	Diputación General de Aragón Dr. Miguel Carravedo mcarravedo@aragob.es	*officinalis* *acutifolius* *maritimus*	1 5 1	No
		Polytechnic University of Valencia Dr Fernando Nuez fnuez@btc.upv.es	*officinalis*	1	No
Germplasm Resources Information Network (2006)	USA	Northeast Regional PI Station USDA, ARS, NERPIS Cornell University Plant Genetic Resources Unit Dr. Larry Roberson lrobertson@pgru.ars.usda.gov	*officinalis* *acutifolius* *brachyphyllus* *declinatus* *ologoclonos* *setaceus*	33 1 1 1 1 3	Yes
Institute for Plant Genetics and Crop Plant Research (2006)	Germany	Institute for Plant Genetics and Crop Plant Research Dr. Andreas Graner graner@ipk-gatersleben.de	*officinalis* *acutifolius*	29 1	Yes
Nordic Gene Bank (2006)	Sweden	Nordic Gene Bank Katarina Wedelsback Bladh nordgen@ngb.se	*officinalis*	7	Yes

7.3 Characterization

The crop-specific descriptor lists promoted by the International Plant Genetic Resources Institute (IPGRI) does not include asparagus. This motivated Cross and Fallon (1996) to propose an asparagus descriptor list. We believe that this list may be

enhanced by adding some attributes as ploidy level, number and diameter of stems, and density of phylloclades from UPOV (1990).

8 Integration of New Biotechnologies in Breeding Programmes

8.1 *In vitro* Culture

In vitro culture techniques were associated with asparagus breeding since its early beginning, enabling the multiplication of selected parent plants of hybrids and facilitating the derivation of homozygous genotypes. We are not going to deal with the enormous bibliography regarding protocols to enhance efficiency of *in vitro* culture outputs in asparagus, since it would deserve a specific chapter in a book of '*in vitro* culture contributions to plant breeding'. However, we will just mention attempts in which, by means of *in vitro* culture as a selective environment, protoplast-derived somaclonal plants with resistance to *Fusarium oxysporum*, when grown in greenhouse, were obtained (Dan and Sthepens, 1995); and regenerated plants from herbicide (chlorsulfuron) resistant cell colonies were advanced (Ganeshan et al., 1999).

Also protocols were developed in *A. officinalis* (Dan and Stephens, 1991 among others) and in *A. densiflorus* cv S*prengeri* (Benmoussa et al. 1997) in order to obtain protoplast and regenerated plants, which are prerequisites for a successful *Fusarium sp*. resistance gene transfer *via* somatic hybridization.

8.2 Molecular Markers and *M* locus

Since the first molecular marker studies in asparagus, attempts were conducted in order to facilitate the early identification of genders related to *Y* or *M* locus for maleness (see section 2.3 for model of inheritance of sex). This would accelerate the selection of supermales in selfed progenies of hermaphrodite plants, facilitate the identification of male doubled-haploids discriminating also somatic cell (*XY*) regenerated male plants from those of microspore origin after in vitro anther culture, and provide evidence of pollen contamination in all-male hybrid seed production.

The first association found between malate dehydrogenase locus and sex-determining gene with a 19.8 recombination frequency (Maestri et al., 1991) was used by González-Castañón and Carbajal-Carcedo (1996) to identify doubled-haploids raised from microspore.

Restivo et al. (1995) found a restriction fragment length polymorphism (RLFP) marker linked at 6.9 cM, which enabled Biffi et al. (1995) a gender prediction with a 93% precision. Later, as the polymerase chain reaction (PCR) markers became preferred, Jiang and Sink (1997) found two random amplified polymorphic DNA (RAPD) and derived sequence characterized amplified regions (SCARS) markers linked at 1.6 cM of the *M* allele in coupling phase, which would only discriminate male from females. Spada et al. (1998) extended the studies including also amplified fragment length polymorphism (AFLP), finding a 3.2 cM distanced dominant inherited marker and Reamon-Büttner et al. (1998) found three AFLPs, which could

be scored codominantly, linked at 0.5, 0.7, and 1 cM from the *M* locus. Then Reamon-Büttner and Jung (2000) derived a sequence-tagged-site (STS) marker which was also inherited codominantly and did not recombine with the *M* locus in five screened populations. Recently, after establishing a BAC (bacterial artificial chromosome) genome library, Jamsari et al. (2004) constructed the first-generation physical map around the sex locus (*M*), deriving a codominat PCR-marker.

8.3 Genetic Map

Nowadays genetic maps are conceived as different molecular markers linked to loci of interest that can be used in marker-facilitated breeding programs, identifying loci involved in agronomic characters and in plant disease resistance. All linkage groups should be covered with a saturation as high as possible.

In asparagus genetic maps were originally presented for isozyme markers with four detectable linkage groups (Maestri et al. 1991). Then studies were extended to RFLP markers revealing seven identified linking groups and a covering length of up to 402 cM (Lewis and Sink, 1996); and also to RFLP, RAPD and AFLP markers, integrating ten linkage groups, spanning 721.4 cM, with an average distance between markers of 2.6 cM (Spada et al. 1998). The chloroplast DNA was also mapped by means of RFLPs and some genes encoding photosynthesis-related proteins, rDNAs and tRNAs were localized (Lee et al., 1996).

As we discussed, so far, *M* is the only allele with agronomic importance that has been linked to genetic markers in the nuclear genome. This means that potential use of genetic markers in asparagus breeding is in its infancy. Bulked segregant analysis using AFLPs and simple sequence repeats (SSR) was proposed to search disease resistant gene(s) (Delaitre et al., 2005).

A promising attempt has been conducted by (Roose et al., 2005) in order to find AFLP markers linked to quantitative trait loci (QTL) for green spear quality, mean spear weight, and marketable yield out from some segregating populations from American x European origin crosses; however, no matching was found for significant QTLs along populations. In a way to enhance the consistency of QTLs in further evaluations, the use of more repetitions and an increased population size were proposed (Roose et al., 2005).

8.4 Transgenesis in Asparagus

Expression of transgenic genes in regenerated asparagus plants was successfully achieved by *Agrobacterium* mediated transformation (Delbreil et al., 1993) and microprojectile bombardment (Cabrera-Ponce et al., 1997). Transformation by electroporation could also be effective since transgenic gene expression was observed in protoplast-derived micro-colonies and callus (Mukhopadhyay and Desjardins, 1994).

A soon as these techniques became available, Conner and Abernethy (1996) pointed out some difficulties that may arise when integrating genetic engineering with asparagus breeding. One of the problem is the impossibility to rise selfed progenies out of the transformed plants (which are normally heterozygous for the

transgene) in order to secure homozygous state for the gene of interest. The other inconvenience is the heterozygous nature of many of the parents of selected hybrids, which would be the targeted genotype to transform. These impediments will be circumvented when the targeted genotype with incorporated transgenes is suitable for clonal multiplication via micropropagation, limiting the first generation of transgenic asparagus to clonal cultivars (Conner and Abernethy, 1996).

If the envisaged transgenic cultivar is seed-grown, the strategy may be to derive doubled-haploids from anther culture of transgenic male plants, but this will be limited to the cases when the targeted genotype is already a doubled haploid homozygous (will segregate only the transgene). Whatever the approach be, special careful should be placed to minimize the frequency of tissue culture-induced variation among the transgenic plants.

Garrison and Chin (2005) envisaged some genes already isolated that could be potentially transferred into asparagus for herbicide tolerance or beetle resistance. The introduction of disease resistance must await the isolation of the proper genes. In any case, consumer acceptance of cultivars enhanced via genetic engineering must precede their introduction.

9 Seed Production

The production of hybrid seed with high germination, vigour, and genetic purity is important for a high value perennial crop such as asparagus. After reviewing the vast literature in asparagus we only found in Walker et al. (1999) a complete guideline for seed production, from which we will try to resume the most important points.

9.1 Site Selection

Ideal location should have frost-free growing season of more than 180 days, 250-500 mm annual rainfall, low frequency of severe storms, hails and high winds. It also must be free from difficulties in the control of perennial weeds and not previously planted with asparagus. The isolation from commercial asparagus fields should be at least 2 km and from any volunteer plant at least 300 m (Ellison, 1986).

9.2 Establishment of Parental Material

The arrangement recommended is a single male row intercalated every four rows of female plants in a plantation grid of 1.5 to 1.8 m between rows and 0.6 to 0.9 m within plants in the row.

9.3 Pollination Management

Spears of the earliest parent can be removed in order to maximize blossom coincidence and seed production. The placement of honeybees at a rate of 2-4 hives per acre since first female flowers open is recommended. Hives should be removed

in the late summer to avoid pollination in the last stalk flushes, which may not complete berry and seed development prior to the first frost.

9.4 Seed Genetic Purity

In all-male seed production blocks contamination could be from pollen of volunteer male plants or eventually from seeds of volunteer female established by seeds brought in by birds.

In dioecious cultivar seed production blocks volunteers could be of either sex. In all instances efforts should be taken in eliminating volunteers as early as they are emerging.

Selective herbicides as linuron have excellent control in seedling stage only. If volunteers escape this stage the may be difficult to rogue out unless mechanically. During fruit harvests caution should be placed in avoiding berry dropping.

In all-male hybrids seed lot samples, multiplication of the percentage of female plants by two provides an indication of the percentage of genetic impurity. For certain dioecious and all-male materials specific markers as isoenzymes (Lallemand et al. 1994), RFLPs and RAPDs (Roose and Stone, 1996) and RAPDs (Khandka et al., 1996; Jaag et al., 1998) were found and could be used to check genetic purity.

9.5 Pest Management

Effective weed and insect control is essential in seed blocks along years. Caution should be taken in the insecticide used to avoid bee mortality. If frequent rains or heavy dew occur preventive fungicides application should be conducted.

9.6 Seed Harvest

Large-scale seed harvesting can be conducted by a modified grain combine harvest machine adjusted to remove berries from the foliage in the field or by stationary threshing equipment, which requires hand cutting and transporting. The former has the disadvantage of depositing some seed back onto the field.

Berries have high moisture at harvest and caution should be taken because respiration heat can be elevated when held in piles. Harvested berries are machine crushed, and the pulp/seed mixture is water washed, and floated low-density pulp separated from seeds. Seed moisture should be lowered to 12-13% prior cleaning, storing and packing.

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Onion

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1 Introduction

Onion (*Allium cepa* L.) is since ancient times a valuable vegetable crop for people all over the world. In this context a mural from Egypt, dated approximately 3000 BC, depicts already images of onions. Therefore it can be inferred that onions were already an important food source for the people from the Ancient Egypt. The word "onion" is derived from Latin and means "large pearl". The onion was compared to a pearl not only for its shape but also for its highly valuable nutritional quality.

Nowadays one can find onions with different colours, tastes and bulb shapes, making the crop an important ingredient in all types of dishes around the world. Onions are mostly fried, stewed or baked before consumption; however they are also eaten raw, especially sweet onions which have a mild taste. Furthermore onions are processed into onion oil or powder and used to flavour a variety of products.

On the list of worldwide cultivated vegetable crops, onions rank second only preceded by tomatoes (FAOSTAT, 2006). Onions are grown all over the world, and as the crop is daylength sensitive several onion types exist depending upon the latitude they grow. Table 1 illustrates the worldwide onion production in 2006. On a worldwide scale around 58 million Metric tons (Mtons) are produced annually. China is by far the top onion producing country in the world, accounting for approximately 46% of the world's onion production, followed by India, USA, Turkey, Iran, Pakistan, and Japan. The worldwide onion exports are estimated around 4 million Mtons. The Netherlands is the world's largest onion exporter with a total of around 800.000 Mton, which is ca. 80% of its total annual production. In spite of the large amount of onions produced and processed every year, the daily consumption of onions per person is relatively low. In Japan, the per capita amount of onions purchased in 2000 was ca. 4.8 kg. However, this number does not include the amount of onions consumed from processed food and meals served at restaurants.

Health beneficial properties are frequently ascribed to onions (and even more to garlic); however the evidence for this is often conflicting (Koch and Lawson, 1996; Keusgen, 2002). Especially in the area of cardiovascular disease, and to a lesser extent cancer, claims have been made, however still we do know too few about the action *of Allium* health beneficial compounds in the human body and its effects on human health (Kik et al., 2001a, 2005). As health promoting compounds in Alliums, organo-sulphur species, flavonoids and fructans have been suggested. The first indicated compounds, the organo-sulphur species, give *Allium* species like onion, garlic and leek, their specific taste and smell.

Onion breeding and genetics has undergone large changes, as so many crops, in the last two decades, as amongst others molecular markers, DNA sequencing, chromosome painting interspecific hybridization and genetic transformation made it possible to analyze the genetic make-up of the species to a far larger extent then previously was possible. In this review the current state of the art will be described and future research directions indicated.

Table 1. The present situation of onion production in the world (FAOSTAT, 2006).

	Onion, Dry			Onion & shallot, Green	
	Planted area 10^3 ha	Production 10^3 Mt		Planted area 10^3 ha	Production 10^3 Mt
World	3,181	57,910	World	231	4,671
Continent			Continent		
Africa	315	5,132	Africa	39	491
North & Central America	109	4,351	North & Central America	46	1,149
South America	149	3,447	South America	14	107
Asia	2,175	36,617	Asia	114	2,468
Europe	426	8,129	Europe	12	215
Oceania	6	233	Oceania	6	242
Main production country			Main production country		
1. China	901	19,047	1. Mexico	44	1,131
2. India	530	5,500	2. South Korea	28	750
3. USA	67	3,700	3. China	22	746
4. Turkey	78	2,000	4. Japan	23	500
5. Pakistan	128	1,765	5. New Zealand	6	242
6. Russia	125	1,640	6. Nigeria	11	220
7. Iran	45	1,450	7. Turkey	22	220
8. Egypt	43	1,302	8. Tunisia	6	138
9. Japan	23	1,200	9. North Korea	8	97
10. Brazil	57	1,059	10. Ecuador	12	93

2 Origin and Domestication

Onion is a species which belongs to the genus *Allium*. This genus consists of around 750 species which are almost entirely distributed on the Northern hemisphere. The majority of the *Allium* species can be found in Eurasia and a smaller part in America. The genus is divided in a number of subgenera and depending upon the author five (Hanelt, 1990) or eleven (Fritsch and Friesen, 2002) are distinguished. Members of the genus *Allium* have a growth preference for open, dry and sunny sites in humid and arid climates. The majority of the species can be found in the region which stretches from the Mediterranean basin in the West to Central Asia in the East.

Although onion is not present anymore in the wild, its closely related wild relatives still can be found in the region in between Iran and Mongolia (Hanelt, 1990). Therefore many botanists believe that onion originates from this region. Findings from ancient Egyptian tombs suggest that the history of onion may date back to at least 3200-2800 BC. Onion cultivation was introduced in India around 600 BC and Greeks and Romans wrote about onion and garlic around 400-300 BC. From here the onion was introduced to the rest of Europe and subsequently to the Americas in the 17th century and Asia in the 19th century (for a review see Fritsch and Friesen, 2002). The spread of onion over the European continent is believed to have taken place via two routes namely (1) the Northern Europe route covering Northwestern India, Afghanistan, Uzbekistan, Romania, and Yugoslavia; and (2) the Southern Europe route covering Spain, France, Italy, and other countries in the Mediterranean region. Onions that spread through the Northern Europe route during medieval times have eventually given rise to long-day onion varieties that are known for their characteristic pungent taste. On the other hand, those that spread via the Southern Europe route have given rise to short-day onions, which are relatively new varieties that are whiter in colour and milder and sweeter in taste. At some point in the 16th century, both the short-day and long-day varieties were introduced in North America. Subsequently, these varieties were further improved through breeding, leading to the creation of a wide range of ecologically diverse cultivars and the invention of different cultivation techniques. India, which is located relatively close to the regions where onions are believed to have originated, also has a long history of cultivating and eating onions. However, in the more eastern part of Asia, such as China, the consumption of onions has remained relatively low over time. In fact, bunching onions, which are considered a staple in Chinese cooking, have always been more popular than onions in East Asia.

3 Varietal Groups

Onion is a biannual predominantly cross-fertilizing diploid species ($2n = 2x = 16$) in which a strong inbreeding depression is present. There is not an unanimous view on the intraspecific classification of *A. cepa*, because of the large variation present within its genepool which is also due to interspecific hybridization. For an overview concerning the ideas about this subject the reader is referred to Friesen and Fritsch (2002). In this chapter we will use the classification of Hanelt (1990) which is based

to a large extent on the ideas of Jones and Mann (1963). The Hanelt classification is easy to use and has practical advantages over other more taxonomically based classifications. In the Hanelt classification, the species *A. cepa* is subdivided into two large groups, namely the Common Onion group and the Aggregatum group, and one smaller group namely the Ever-ready Onion group. The presence of interspecific hybrids which sometimes resemble members of the three *A. cepa* groups are excluded in the Hanelt classification and are seen as a separate group of hybrid taxa. As these hybrid taxa are of interest for scientific as well as applied purposes these taxa are described briefly. Two types of hybrid taxa have been described which have *A. cepa* as a parent, namely *A.* x *proliferum* and *A.* x *cornutum*. The *A.* x *proliferum* type consists of material that originates from hybridizations between members of the *A. cepa* Common Onion group and *A. fistulosum*. These types are known as top onion, tree onion, Egyptian onions and catawissa onions. Other material originates from crosses between members of the *A. cepa* Aggregatum group and *A. fistulosum*. *A.* x wakegi, a shallot which is grown in tropical conditions, is an example of such a successful cross. *A.* x *cornutum* is a triploid viviparous onion of which two chromosome complements originate from *A. cepa* whereas the other parent is yet unknown. Examples of this type of hybrid taxa are 'Pran' which is cultivated in NW India (Havey, 1993) and 'Ljutika' which is cultivated in SE Europe (Puizina and Papes, 1997).

3.1 Common Onion Group

The bulb of onion is formed by the thickening of leaf-bases a short distance above the stem. Outer leaf-bases are thin, fibrous and dry, variously coloured, and form the protective bulb-coat. Mature bulbs have a globose to ovoid shape, are up to 15 cm in diameter, and are very variable in size, colour and weight. The bulbs are normally single, and plants reproduce from seeds or from seed-grown sets. Nowadays onions are cultivated almost worldwide at latitudes between 5-60° in both hemispheres. The crop includes hundreds of open-pollinated traditional and modern F_1 hybrid varieties (Fritsch and Friesen, 2002). Genetic erosion is thought to have a major impact on the variation in this group, because of the widespread introduction of highly uniform and productive F_1 hybrid varieties. Nonetheless, significant diversity can be still be found in the Central Asia (North India, Pakistan, European and Middle Asian republics, the former Soviet Union) and the Mediterranean basin (from Spain in the west to Turkey in the east) (Astley et al., 1982; Bosch Serra and Currah, 2002). Onion varieties are distinguished and categorized mainly by a range of bulb characteristics (Table 2). Bulb characteristics include skin colour, shape and size. The bulb shape can be oblate, thick flat, obovate, ovate, oval, torpedo, etc. Skin colours can be white, yellow, brown, red and intermediates between these colours. A descriptor list for onion has been developed and currently this list is used for classifying onion accessions from all European countries (Gass et al., 1995).

Table 2. Classification of principal varieties in LD, ID and SD bulb onions (data source: Oregon state university, http://oregonstate.edu/dept/hort/233/onion.htm, with some minor modifications). A larger version of this Table is available together after the color insert at the beginning of the book.

Category									Cultivar
I	II	III	IV	V	VI	VII	VIII	IX	
Yellow or brown skin	Brown skin, oblate								Australian Brown
	Yellow skin	Thick flat to medium oblate	Thick flat, light straw-yellow scales, strong flavor						Yellow Bermuda
			Medium oblate, pinkish-yellow scales, strong flavor						Ebenezer
		Not thick, flat to medium oblate	Deep oblate	Scales light to medium yellow					Early Yellow Globe
				Scales medium brownish-yellow					Mountain Danvers
			Not deep oblate	obovate (flattened base)					Ohio Yellow Globe
				Not obolonte	Ovate (flattened upper half)				Early Grano
					Not ovate	Round, medium brownish-yellow scales			Yellow Globe Danvers
						Oval	Medium size	Scales medium yellow and medium thick	Southport Yellow Globe
								Scales deep yellow and thick	Brigham Yellow Globe
							Large size, scales brownish-yellow, flavor mild		Sweet Spanish
Not yellow or brown skin	White skin	Flat, large diameter, mild flavor							Crystal Wax
		Not flat	Thick flat to oblate	Thick flat, early maturing					White Creole
				Oblate, late maturing					White Portugal
			Deep oblate to round	Medium size, strong flavor					Southport White Globe
				Large size, mild flavor					White Sweet Spanish
	Red or purple skin	Mild flavor, soft flesh	Thick flat, large size	Torpedo or long oval					California Early Red
		Strong flavor, firm flesh	Thick flat, large size						Italian Red
			Not thick flat	Thick flat to oblate, buff red					Red Wethersfield
				Round to oval, dark purplish red					Red Creole
									Southport Red Globe

3.2 Aggregatum Group

The bulbs of this group are smaller than the common onion because they form an aggregated cluster of lateral bulbs. Most varieties in this group are propagated asexually by bulb multiplication. Jones and Mann (1963) divided this group into two sub-groups on the basis of their bulb-forming phenotypes, namely a. shallots and b. multiplier or potato onions. However Permadi and Van der Meer (1993) did not make this subdivision and suggested that this group includes the ever-ready onion (UK), the Russian vegetatively-propagated onion, the 'Utrechtse Sint Jansui' (the Netherlands), 'Griselle' (France, see Maaβ, 1996), 'Pran' (India, see Havey, 1991), 'Ljutika' (Croatia, see Puizina and Papes, 1996) together with shallot and multiplier or potato onion.

Shallot seems to be derived from common onion by selection among naturally occurring variants (Bailey, 1949) and the first reliable records of its existence date back to 12th century in France (Permadi and Van der Meer, 1993). This crop is now found from the equator to both polar circles. The shallot is more important than common onion in the tropical lowlands at latitudes between 10˚N and 10˚S and is cultivated mainly in Southeast Asian and African countries. Genetic variability in shallots was studied, using on isozyme and/or DNA markers, in Indonesia (Arifin et al., 2000), Vietnam (Phuong et al., 2006a) and their surrounding countries (Arifin and Okubo, 1996; Phuong et al., 2006b). The so-called potato or multiplier onion is also grown in South-East Asia. According to Permadi and Van der Meer (1993), they

differ from shallots in their larger bulb size, their often somewhat flattened shape, and usually having fewer daughter bulbs which remain enclosed by the skin of the mother bulb for a longer period than in shallot.

The 'Griselle' is one of the varieties of 'grey shallot' which has been cultivated in France for a long time and which is highly valued for its taste. The results of RAPD and GISH investigations clearly showed that the grey shallot belongs to *A. oschaninii* and not to *A. cepa* (Friesen and Klaas, 1998). Puizina and Papes (1997) analyzed via conventional genetic analysis the viviparous triploid onion varieties, 'Ljutika' and 'Pran', and suggested that both originated via the backcrossing of an interspecific hybrid (probably between *A. cepa* and *A. fistulosum*) to another parental species. Furthermore Friesen and Klaas (1998) showed via GISH that part of the triploid onion material has a segmental alloploid genetic background possessing an additional parental genome from another species closely related to *A. cepa*.

3.3 Ever-Ready Onion Group

According to Hanelt (1990) and Fritsch and Friesen (2002), who based themselves also on detailed descriptions of Stearn (1943) and Jones and Mann (1963) this third group varietal group of *A. cepa* can be distinguished from the other two by its strong vegetative growth and its lack of dormancy, therefore its bulbs or leaves are available year-round. The ever-ready onion was grown in the UK (mainly in gardens) until the 1950's and was used as a salad onion. Recently Friesen and Klaas (1998) showed that, ever-ready onion is more closely related to common onion than to shallot and *A. vavilovii*.

4 Genetic Resources

In the first large overview on Alliums namely 'Onions and their Allies' written by Jones and Mann in 1963 the topic of genetic resources is not mentioned. Although nowadays genetic resources is an important issue, in the first half of the 20th century this was not the case. Genetic resources became worldwide an important topic in the late sixties, although the first signals of the action of genetic erosion on crops were already observed decades earlier by Harlan and Vavilov (Pistorius, 1997). Astley et al. (1982) were the first that made an overview of global *Allium* genetic resources. Their report was focused on cultivated *Allium* species. In this report they identified the major *Allium* collections worldwide and the species and numbers of accessions per species per collection. Also they included a first draft of an *Allium* descriptor list and a list of collecting priorities. In total 9078 cultivated *Allium* accessions were reported to be present in the various collections worldwide and the number of onion accessions was by 73% by far the largest. The collection of local/modern cultivars and landraces of *Allium cepa* (dry bulb onions and shallots) was considered as the major future priority in view of the considerable genetic erosion present for this crop species. In 1990 Astley described the present status on the conservation of *Allium* genetic resources and focused on discussing *ex situ* conservation: characterization, evaluation, documentation and utilization of collections. Furthermore he indicated

that wild *Allium* taxa are seriously underrepresented in global collections and those distribution areas of species are not adequately covered. In 1998 Cross wrote a review paper on global resources of vegetables and mentioned that '*cultivated Allium species are well collected (12,382 accessions), but primary and secondary centres of biodiversity are poorly sampled. Therefore the comprehensiveness of Allium collections worldwide is possibly inadequate*'.

Currently around 27,000 *Allium* accessions are held in genebanks worldwide (www.ipgri.cgiar.org/germplasm/dbintro.htm). However one must consider this number as an upper limit as the percentage of duplications within and between *Allium* collections is unknown, misclassification is not accounted for and also availability of accessions is unclear in many cases. Especially the percentage of duplication can be of influence on the total number of accessions held as for example in lettuce 60% of duplication was found in the collections of the major collection holders (Van Hintum and Boukema, 1999). In table 3, the number of *Allium* accessions is indicated that are present in worldwide collections. As can be seen clearly onion/shallot is represented most (46.7%), followed by garlic (16.7%) and leek (7.9%). It could be suggested that for these species collection efforts should only be directed towards the collection of accessions in areas where sampling has not been intensive.

Table 3. Number of accessions per species and occurrence (%) of species in worldwide *Allium* collections (data from IPGRI database; www.ipgri.cgiar.org/ germplasm/dbintro. htm).

species	number of accessions	%
A. cepa	12740	46.7
A. sativum	4560	16.7
A. porrum	2148	7.9
A. fistulosum	951	3.5
A. tuberosum	434	1.6
A. schoenoprasum	274	1.0
A. nutans	95	0.3
A. chinense	27	0.1
wild relatives	6073	22.2
total	27302	100.0

For all the other 750 *Allium* species, edible and wild, genetic resources are not sufficient and collection missions are clearly needed. For onion, wild relatives are very important, as most probably onion itself does not carry important disease and pest resistance genes and other quality (e.g. rooting system) genes. To delimit the number of *Allium* species to be taken into account for crossability analysis, phylogenetic insights are of importance. In this respect the publication of Hanelt (1990) and the recent publication of Friesen et al. (2005) are of interest. The main difference between the two papers is that sections identified in the subgenus Rhizirideum by Hanelt were raised to the subgenus level by Friesen et al. to circumvent polyphyly. In the Hanelt phylogeny, species from subgenus Rhizirideum

(around 225 species) can be considered to be potentially exploitable. In the Friesen phylogeny this number could possibly be delimited to 30 species as this number of species occurs in subgenus Cepa. However Friesen et al. placed *A. roylei*, a species which can be hybridized with onion (for review see Kik, 2002), in subgenus Polyprason, and this leads to the conclusion that one can not delimit oneself only to subgenus Cepa for the identification of wild crossable relatives of onion. Consequently we are still in the position which Hanelt in 1990 described with respect to which species can be potentially exploited for onion breeding via sexual hybridization. Van Raamsdonk et al. (2003) reviewed the existing literature which species can be used for onion breeding and they showed that only a few species can be crossed directly with onion, namely *A. cepa*, *A. vavilovii*, *A. galanthum* and *A. roylei*. These species can be considered as species from the primary genepool of onion (genepool concept: Harlan and de Wet, 1971). The secondary genepool is at least composed of *A. fistulosum* and its *progenitor A. altaicum*, as Khrustaleva and Kik (2000) convincingly showed that *A. roylei* can act as a bridging species between onion and *A. fistulosum* / *A. altaicum*. The tertiary genepool consists of *A. pskemense* and *A. oschaninii* and another twenty species from the subgenera Cepa, Reticulatabulbosa, Polyprason and Anguinum (sensu Friesen et al., 2005). In table 4 an overview is given of the number of accessions per species present in the different onion genepools. From this table it can be concluded that although the onion germplasm is reasonably available, the germplasm of the other wild relatives are only present in limited numbers. Given the fact that a high percentage of duplication is widely occurring in germplasm collections, it is clear that collection missions or *in situ* conservation measures should be carried out to safeguard these important genetic resources. This is not only true for the species indicated in table 4, but even more for the species in table 5 in which an overview is given of the species of subgenus Rhizirideum (sensu Hanelt) present in genebanks worldwide. It appears that around 60% of the total number of species from this subgenus (sensu Hanelt) is present in worldwide collections. Therefore collection missions are needed to sample the other 40 percent.

Table 4. The onion genepools; number of accessions based on the IPGRI database (www.ipgri.cgiar.org/ germplasm/dbintro.htm).

genepools	number of accessions
primary	
A. cepa	12740
A. vavilovii	20
A. galanthum	34
A. roylei	4
secundary	
A. fistulosum	951
A. altaicum	121
tertiary	
A. pskemense	21
A. oschaninii	41

Table 5. Number of species present in subgenus Rhizirideum (sensu Hanelt (1990)), and the number of species/accessions present in genebanks worldwide. Between brackets very rarely sampled species are indicated.

subgenus (sensu Friesen)	number of species/subgenus	number of species in genebanks	number of accessions in genebanks
Cepa	22 (8)	18 (2)	14641
Reticulatabulbosa	55 (18)	34 (6)	393
Polyprason	61 (14)	29 (5)	252
Rhizirideum	20 (11)	19 (1)	681
Butomissa	4	3	615
Anguinum	5 (5)	4 (2)	56
Cyatophora	3 (1)	2	75
	170 (57)	109 (16)	16723

Very important in future onion genetic resources is that a serious effort should be undertaken to determine which *Allium* species belong to which part of the genepool in order to utilize the onion genetic resources in a better way. This information can be used to develop better *in situ* and *ex situ* strategies for the onion genepools. It could be suggested in this respect, that species belonging to the primary and secondary onion genepool will be maintained in future predominantly via *ex situ* management whereas species from the tertiary genepool will be managed predominantly via *in situ* methods.

5 Major Breeding Achievements

5.1 F_1 Hybrid Breeding

5.1.1 Fundamental Aspects of F_1 Hybrid Breeding

Male sterility in onions was discovered for the first time in 1925 in the cultivar Italian Red which was grown in California (USA; for a detailed historic overview see Jones and Mann, 1963). This type of male sterility was determined by an interaction between the nucleus and the cytoplasm and its genetic basis proved to be simple, namely on the nuclear side one restorer gene with two alleles (M and m) and on the cytoplasm side two types of cytoplasms: N(ormal) and S(terile) (Jones and Clarke, 1943). This type of male sterility is also known as cytoplasmic male sterility or CMS (for review Kaul, 1988). The first CMS source discovered is nowadays known as CMS-S. A different source of CMS in onions, namely CMS-T, was identified by Berninger (1965) in the French cultivar Jaune paille de Vertus. Schweisguth (1973) analyzed the genetic basis of this type of CMS and showed that male sterility in this case was restored by a dominant allele at locus A or dominant alleles at two complementary functioning loci, the so-called B and C loci. Currently both types of CMS are used in commercial F_1 hybrid seed production, although the CMS-S source

is probably used most frequently worldwide as only in Dutch and Japanese cultivars CMS-T was discovered (Havey, 2000).

De Courcel et al. (1989) showed via molecular marker analysis that there are two groups of cytoplasms namely the S and the M group. In the M group two subgroups were observed namely the N and the T group. Within these groups variability was found, which makes the development of molecular markers which can distinguish both groups difficult. However in the S cytoplasm group no or very little variation was discovered (De Courcel, 1989; Holford et al., 1991a), which has led to the development of PCR markers that can make a distinction between the S and the other two cytoplasm types (Havey, 1995). Furthermore it was shown by Havey (1993) using restriction analysis that the frequency of the S cytoplasm can differ to a large extent among onion populations worldwide. As the S cytoplasm is so deviant from the other two cytoplasms Holford et al. (1991a) hypothesized that this cytoplasm originated from interspecific cross (alloplasmic CMS), whereas the T cytoplasm originated from a mutation of the N cytoplasm (autoplasmic CMS). In this context Havey (2000) proposed that the S cytoplasm was introduced into onions via 'Pran' a viviparous triploid onion (*A.* x *cornutum*) and that the N and T cytoplasms originated from a progenitor of the M cytoplasm group. In this respect he proposed that *A. vavilovii* might be this progenitor. Microsporogenesis in both CMS sources takes place in a different way: CMS-S proceeds normally through meiosis whereas CMS-T behaves abnormally. In CMS-S premature breakdown of the tapetum at tetrad stage, hypertrophy of the tapetum at dyad stage and delayed retention of the tapetum has been observed with in one single inbred line (Holford et al., 1991b).

The frequency of the restorer allele for CMS-S can also differ among populations (Havey, 1995). Especially in tropical populations where the frequency of the M allele is close to 100% it can become a problem for the discovery of maintainers which are of pivotal importance for the development of an F_1 hybrid seed program (Van der Meer, 1994). The restorer locus for CMS-S has been located on chromosome 2 (Martin et al., 2005). A tightly linked (0.9 cM) molecular marker to this restorer locus, which can facilitate the breeding process of new onion cultivars, has also been found. Unfortunately this marker was not in linkage disequilibrium with the restorer locus (Gokce and Havey, 2002). For CMS-T no research has been published until present which identifies markers tightly linked to the A locus.

In breeding practice nowadays both CMS S and T are being viewed upon as having on the nuclear side one restorer locus. It seems therefore that breeders have selected in practice against the complementary B and C restorer loci of the T cytoplasm. Also it is known that CMS-S A lines are not producing any pollen under high temperatures, so they are thermostabile. However for CMS-T A lines thermolability has been reported (Van der Meer and Bennekom, 1969) although at that time they thought that they were dealing with CMS-S. Strong selection by breeders against female fertility in commercial CMS-T A lines has currently considerably improved this situation. As the vulnerability of crops could increase when its genetic basis is too small (Levings, 1990), a new CMS source originating from *A. galanthum* was recently introduced into onions (Yamashita and Tashiro, 1999; Havey, 1999). This CMS source has similar seed yields as the S cytoplasm and

has no restorers. In the coming years it will become clear if, and to what extent, this CMS source will penetrate into the onion F_1 hybrid market.

5.1.2 Practical Aspects of F_1 Hybrid Breeding

The first commercial F_1 hybrids were brought to the seed market in the early fifties in the USA. Since then F_1 hybrids increased their share every year on the global onion seed market. Although this process went much faster on the temperate onion seed market compare to the tropical seed market. In the late sixties the first F_1 hybrid showed up on the Dutch seed market (Kik et al., 2001b). Nowadays the global temperate onion seed market consists over 90% of F_1 hybrids, however on the onion seed market for the tropics the frequency of OP varieties is still around 50-60%; Kik et al., 1998).

The exploitation of heterosis, the increase in uniformity, the relative ease to combine traits and the built-in variety protection are frequently reported advantages of F_1 hybrids. It is evident that the latter two reported advantages are of considerable importance in F_1 hybrid breeding; however, the exploitation of heterosis and the increase of uniformity are less evident. This is because we can look upon F_1 hybrid onion breeding as an advanced type of S_1 family and mass selection. The breeding lines developed in long day onion cultivars are mostly two sometimes three times inbred and are subsequently maintained by mass selection, therefore these lines do still have considerable within line genetic variation. For short day onion cultivars this is even worse as inbreeding is applied in most cases only once. Therefore one can understand that uniformity in F_1 hybrid varieties is not necessarily higher compared OP varieties which have been subjected to strong positive mass selection. To explain the sometimes observed increased uniformity in F_1 hybrids over OP cultivars, it has also been hypothesized that the exclusion of selfing in F_1 hybrids may reduce variability in these types of cultivars (cf. leek; Smith and Crowther, 1995). However the magnitude of this effect is not clear as little research has been carried out in this respect. Also the presence of strong heterosis in onion is doubtful, as no large differences occur in mean yields between OP and F_1 hybrids were observed in long day onion cultivars (Van der Meer, 1994). In this context Werner et al. (1988, 1990) showed that the genetic basis of heterosis in onions is probably based on the dispersion of partially dominant alleles rather than on overdominance. All in all, the advantages of F_1 hybrids over OP based cultivars for seed companies is the in-built variety protection which forces farmers to buy seed periodically and the more easy way of combining traits.

5.2 Disease and Pest Resistance

Onions are threatened by a large number of diseases and pests. Only in a few cases disease and pest research resulted in practical applications to control them (Schwartz and Mohan, 1995). For the onion fly (*Delia antiqua*) research in the seventies and eighties of the previous century resulted in the development of a sterile insect technique which proved to be quite successful in controlling this pest (Loosjes, 1976). For other pests no such breakthroughs have occurred yet, also not in the area

of breeding for resistance (Lorbeer et al., 2002). In the case of diseases, IPM programs were developed in the eighties of the last century for downy mildew (DOWNCAST; Hildebrandt and Sutton, 1984; De Visser, 1998) and onion leaf blight (BOTCAST; Sutton et al., 1986). Breeding for resistance led to the development of a complete resistance to downy mildew (caused by *Peronospora destructor*), the introduction of a high partial resistance against *Fusarium* basal rot (caused by *Fusarium oxysporum*) and the introduction of field resistance to pink root (caused by *Phoma terrestris*). As the breeding for resistance to downy mildew, *Fusarium* basal rot and pink root resulted in the development of improved onion cultivars in the last two decades, these diseases will be dealt with in this chapter.

A chapter on biotic resistance in onions can not be written without a reference to white rot (caused by *Sclerotium cepivorum*): a disease which is particularly threatening onion cultivations worldwide due to the intimate relationship of this fungus with members of the genus *Allium* (for review: Entwistle, 1990). Until present the only way to control the disease is the use of soil fungicides and diallyldisulfides (DADS), the latter causing the sclerotia of the fungus to germinate in the absence of *Allium* crops. Breeding for resistance until present has not been a success, as the resistance to the fungus should most probably be based upon the avoidance of the leakage of organo-sulphur compounds from the plants to the soil: a shear impossible task.

5.2.1 Downy Mildew

Downy mildew, caused by *Peronospora destructor*, is a fungal disease in onions which causes every year worldwide considerable yield losses. Until present the disease is controlled in onion cultivations by the use fungicides. In order to reduce the amount of fungicides and thereby their possible environmental impact, an IPM program has been developed entitled DOWNCAST (Hildebrandt and Sutton, 1984; De Visser, 1998). Although such an IPM program proved to be highly beneficial for onion cultivation, the introduction of a complete or high partial resistance to the crop is still desirable. Resistance to this disease which protects the whole plant is not available within the onion germplasm, only a complete resistance to the disease has been reported for the seed-stems of cv. Calred (Jones and Mann, 1963). Furthermore sources of resistance in wild crossable relatives of onion are not available (Kofoet and Zinkernagel, 1990). However in 1990 Van der Meer and de Vries reported that a complete resistance to this disease could be found in *Allium roylei.* Also they showed that both species could be crossed with each other yielding a partially fertile interspecific hybrid. Kofoet et al. (1990) showed that a backcross progeny with onion segregated into a 1:1 (resistant:susceptible) fashion and that the interspecific hybrid was resistant to downy mildew infection. This led them to hypothesize that the genetic basis of the resistance was based on one gene with the dominant allele being the allele carrying the resistance. This locus they called Pd1. In 1992 De Vries et al. (1992a) showed that after analyzing a selfed progeny of the interspecific hybrid that the segregation ration deviated from the expected 3:1 (resistant:susceptible) ratio, suggesting that there was possibly a second resistance gene (Pd2) involved in the resistance which was loosely linked (r=0.32) with the first resistance (Pd1) gene.

Via a bulked segregant analysis (BSA; Mitchelmore et al., 1991) De Vries et al. (1992b), found RAPD markers linked to the downy mildew resistance, the closest being 2.6 cM away from one of the resistance loci. The most closely linked RAPD marker was subsequently converted into a SCAR marker and issued to breeding companies. Later on Van Heusden et al. (2000a,b) mapped the downy mildew SCAR marker on the distal end of chromosome 3 using monosomic addition lines. Furthermore they showed that the genetic basis of the resistance to downy mildew was based on one locus with a skewed segregation: a phenomenon that is found frequently in interspecific crosses. In the coming one-two years commercial onion cultivars with a resistance against downy mildew, originating from *A. roylei*, will most probably be released on the market. All in all, it took ca. 20 years from the finding of an *A. roylei* plant resistant to downy mildew to the introduction of cultivars resistant to the disease. It will be now of great interest to see how sustainable the resistance will be as fungi from the Oomycetes are known to have the capacity to breakdown introduced resistances (see for example lettuce-*Bremia* or spinach-*Peronospora*).

5.2.2 Fusarium Basal Rot

Fusarium basal rot (FBR) is a soil-borne disease caused by *F. osysporum* f. sp. *cepae*. In Japan, the problems with FBR started around 1965 in the Furano area which is located on the North Japanese island Hokkaido. Kodama (1983) discovered that a seed treatment involving the submerging of the seed into benomyl was quite effective, and this discovery allowed to continue the cultivation of onions in areas where the disease occurred ubiquitously. However, complete disease prevention was not achieved by this method, and also phytotoxicity or chemical injury occurred such as the failure of rooting after transplanting. For these reasons, the development of resistant varieties was clearly needed.

In 1967, Gabelman and co-workers succeeded in the development of an A and B line with a high partial resistance. In 1972, an improved version of these lines was released. On the basis of this work a FBR-resistant F_1 variety called ‘Fusario 24’ was released. Hokkaido National Agricultural Experimental Station evaluated ‘Fusario 24’, and showed that in infested fields of the Furano area the cultivar had a high partial resistance against the disease However, ‘Fusario 24’ matured late and did not bulb sufficiently. Therefore it was concluded that the variety is not suitable for Hokkaido. Subsequently a line with a high partial resistance, namely ‘F316’ was extracted from ‘Sapporo-ki’ and crossed with the A line of ‘Fusario 24’. The resulting F_1 hybrid 'Furanui' had almost the same resistance as ‘Fusario 24’ against FBR, and was superior to ‘Sapporo-ki’ in marketable standard bulb size and bulb quality. The cultivar was certified in 1979 as Hokkaido's recommended resistant variety against FBR as the first in Japan. After that other resistant varieties against the disease were developed, like ‘Tsukihikari’ (Tanaka et al., 1987) and ‘Sekihoku’ (Miyaura et al., 1985). In both cases, the seed parent was the A line which was also used to develop ‘Fusario 24’. Using material with a high partial resistance selected from cultivars like ‘Early Yellow Globe’, ‘W52’ or ‘Sappori-ki’, a seed company developed ‘Ohotsuku 1’ (early season cultivar), ‘Kita momiji 2000’ (mid-season

cultivar), ‘Super kita momiji’ (late season cultivar) (Iwata 2005). By using these three types throughout the year, farmers can grow onions from early to late in the year. In 2005, the three types represented ca. 82% of all varieties grown in Hokkaido, and the total area planted with these three cultivars was around 9200 ha. Without those resistant cultivars, onion production in Hokkaido would have been nearly commercially impossible. As a result of global warming the disease nowadays also spreads from (sub)tropical areas into temperate areas and in Western Europe the disease is becoming more important during the last decade and the need for resistant varieties is increasing every year.

Evaluation of onion germplasm for FBR resistance is mostly done via field screenings. However also laboratory assays exist like the seedling test developed by Retig (1970). In this test onion seeds are sown in specifically prepared sand mixed with the fungus and grown at 25°C. Seeds with a high partial resistance develop normally, but susceptible seeds die within a week. However, the sand mixture dries easily, and test results obtained via this method do often not correlate with field test results. Therefore there is still room for improvement as for example the mixing of the sand with Hokkaido's volcano ash to keep the sand mixture wet for a prolonged time (Iwata, 2005). Concerning the genetic basis of the FBR resistance a number of models have been put forward in the past, which range from single nuclear dominant genes via multiple nuclear genes to cytoplasmic genes determining the resistance (for review see Cramer, 2000). Hopefully in the near future the genetic basis of the disease can be elucidated by means of QTL analysis, however such an analysis strongly dependents upon a robust resistance test.

5.2.3 Pink Root

Pink root disease, caused by *Pyrenochaeta terrestris* or nowadays known as *Phoma terrestris*, is predominantly found in (sub) tropical and dry climates. The disease can be identified by the occurrence of pink roots on infected onion plants. Pink root can cause considerable yield losses and therefore the introduction of resistance to this disease in the onion crop was carried out. Pink root resistance breeding activities did not involve the use of alien germplasm. The resistance currently present in the Grano type onion cultivars is not high; however it can give the onion plants protection (field resistance) to the disease. More research to introduce better sources of resistance to this disease is clearly warranted.

6 Current Goals of Breeding

There is not one world market for onions, but rather a number of markets each with its own specific requirements concerning cultivar choice, cultivation method, etc. As onions are daylength dependent, cultivars adapted to a certain longitude can not be grown commercially in other longitudes. Therefore different onion types depending on the longitude they grow can be distinguished, namely long long day (LLD), long day (LD), intermediate day (ID) and short day (SD) onion cultivars. LLD cultivars are grown in Europe north of Paris onwards and in North America in south Canada

and the Northeastern part of the USA. The LD cultivars can be found in Europe between Paris and Barcelona and in the USA from Wisconsin in the east to Montana in the west. ID cultivars are cultivated in Europe between Barcelona and Morocco, whereas this type in the USA grows in Idaho and states on the same latitude. The SD growing area in North Africa is from Morocco to the equator and in America SD types can be found in the state of Texas (USA) and Mexico and more to the south.

The two economically most important global markets for onions are the storage and the fresh markets. These two markets cover around 90% of the existing onion markets. Other minor markets are amongst others the dehydration market (onions with 20-25% dry matter content; grown in California, USA), Japanese overwintering onions (grown in Europe in the Pfalz (Germany), Italy, Hungary, Austria, and Switserland), organic onions, pickling onions, etc. The cultivation areas for the two largest market segments are indicated in Table 6. From this table it can be seen that on a global scale the cultivation areas for both storage and fresh onions do not differ that much. However among continents large differences are apparent. In Europe and the Americas predominantly storage type onions are grown, whereas in Africa and Asia predominantly onions for the fresh market are cultivated. Furthermore in Asia around 1 million hectares of bunching onion (*Allium fistulosum*) is grown.

Table 6. The estimated number of hectares per continent used for the cultivation of storage and fresh onions incl. *Allium fistulosum* (bunching onions) and shallots (H. de Groot, pers. comm.).

Continent	Storage (x 1000 ha)	Fresh (x 1000 ha)
Europe	376	50
Africa	93	222
Asia	875	1300
North & Central America	70	39
South America	100	49
Oceania	6	-
Total	1520	1660

6.1 Storage Market

Onions for the storage market are predominantly grown a. in Europe (North Western part: LLD cultivars of the Rijnsburger type; for example cv. Hyskin and South Western part: ID Recas types), b. in New Zealand (LD cultivars like cv. Pukekohe Longkeeper) and c. Canada and the North East part of the USA (LD onion cultivars like cv. Danvers Yellow Globe), all these onions can be stored around 6-7 months. Furthermore specific types of SD onions can also be stored, but this is only for 2-3 months (India: Red Bombay types). The storage type of onion is used for a variety of purposes but mostly for cooking. The phenotypic appearance of the bulbs of these types of onions is medium size, globe-shaped and mostly yellow-brown. The storage onions develop their bulbs towards the shorter days, so after the longest days which

consequently results in a more reduced growth during bulbing and therefore in more compact and firm bulbs which makes this onion ideally suited for transportation worldwide. In this context, the Netherlands export around 80% of their onions harvested annually (around 800.000 Mtons grown on 18,000 ha) to all parts of the world. The three main breeding goals of these types of onions are yield increase, improvement of skin retention and storage ability (bulb firmness and sprout dormancy). Furthermore breeding for disease (downy mildew, onion leaf blight, etc) and pest (thrips, onion maggot, etc) resistance and internal bulb quality (pyruvate, carbohydrates) are of importance.

6.2 Fresh Market

Onions grown for the fresh market in Europe are cultivated predominantly in the south (Spanish Babosa types). In North America these types of onions are cultivated in California, Arizona, Texas, Georgia and other states on the same latitude (Texas Yellow Grano group). By far the largest part of these onions, grown for the fresh market, is cultivated in Asia and Africa. These types of onions develop their bulbs towards the longest day and are harvested during summer. As bulb development for this type on onions occurs in rather warm conditions bulb development is quick and this results in softer bulbs which can not be stored for a long time. Important current breeding goals in onions for the fresh market are yield increase, single centred bulbs and increase of bulb diameter and a low pungency. The last three traits are of eminent importance for onion slicing (salad onion). Also skin retention and storage ability are of importance however less important then in case of storage onions. Furthermore breeding for disease (pink root, Fusarium basal rot, etc) and pest (thrips, onion maggot, etc) resistance is of some importance although most growers rely on fungicides and pesticides to control diseases and pests in their cultivations. In this respect onion cultivars with a field tolerance to pink root (the PRR types) and Fusarium have been developed in the past.

7 Breeding Methods and Techniques

Three breeding methods will be described via which onion cultivars can be developed, namely mass selection, synthetic breeding and F_1 hybrid breeding. Since a long time mass selection has been practiced in onion breeding, however since the elucidation of the genetic basis of CMS in onions by Jones and Clarke (1943) F_1 hybrid breeding became a reality. The first F_1 hybrid cultivars were commercially produced in the fifties in the USA and it was in the late sixties that the first Dutch F_1 hybrid entered the seed market in the Netherlands. Since the 1980's F_1 hybrid breeding became the dominant method to produce new onion cultivars (for a detailed description of F_1 hybrid onion breeding see Pike, 1986 and references therein). However still new cultivars are being developed using mass selection but this technique is mostly used to develop conventional SD onion cultivars or onion cultivars to be cultivated in organic conditions, as the use of F_1 hybrid breeding without making use of restorer genes is not accepted. Synthetic onion cultivars have

also been developed but this technique is far less used compared to the other two selection methods.

7.1 Open-Pollinating Population-Improving System Using Mass Selection

Although very simple, mass selection is an appropriate breeding technique in open-pollinating population-improvement systems (Figure 1). Mass selection is based only on phenotypic selection. The next generation is build-up by mixing the seeds of the selected individuals of the current generation and subsequently a new selection cycle can start. This procedure can take place a number of selection cycles, mostly less than five, before a new cultivar will be released. All types of onions can be used as basic material for the development of a new cultivar based on mass selection, for example plant material from commercial OP cultivars, fertile F_1 cultivars, native OP cultivars, introduced OP cultivars or elite inbreds.

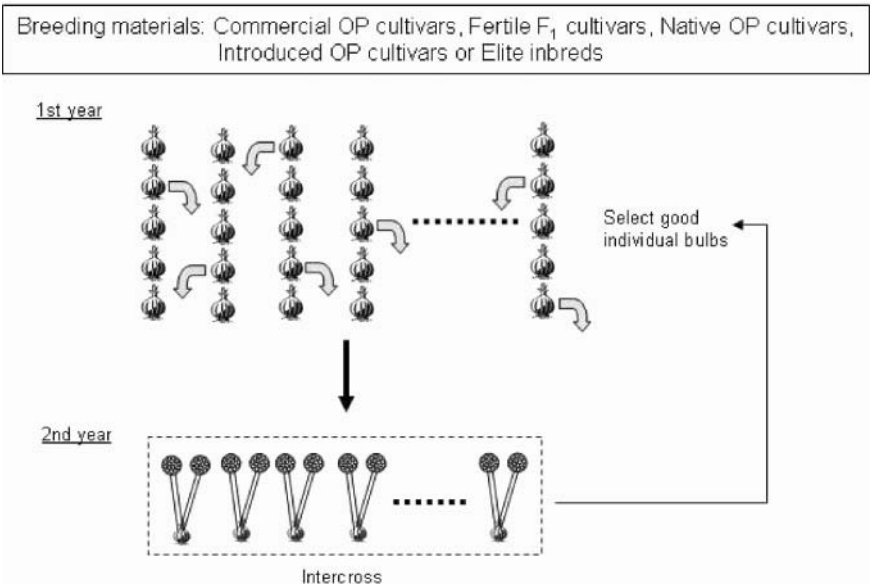


Fig. 1. General breeding scheme for the development of new onion OP cultivars via mass selection.

In old local cultivars raised on farms, mass selection has unconsciously been repeated since ancient times and was probably applied in open-pollinated European onion varieties until the 1970s (Kik et al., 2001b). Since then, the popularity of F_1 hybrids has been gradually spreading all over the world. However, a number of local onion cultivars are still derived from mass selection, for example the breeding of extremely early Japanese overwintering type onion cultivars takes still place via

mass selection. Via this selection procedure high quality cultivars like 'Taka-Nishiki' and 'Hama-Sodachi' have been developed (Figure 2).

Fig. 2. Typical seed production field for an extremely early Japanese onion cultivar.

7.2 Breeding of Synthetic Varieties

Synthetic varieties are predominantly used in forage species like grasses and clovers. These species grow in perennial pastures must withstand large environmental fluctuations. The synthetic variety is thought to be suited for such environments given its broad genetic basis as mostly 5-10 highly heterozygous parental clones form the basis of a synthetic cultivar. Also for onions synthetics exist although far less compared with OP's and F_1 hybrids. The synthetic onion variety is raised by reciprocal crossing between genotypes selected for their ability to combine, and it is maintained by natural crossing. Parental lines for the synthetic variety include commercial OP cultivars, fertile F_1 cultivars, native OP cultivars, introduced OP cultivars or elite inbreds, as shown in Figure 3. S_1 family selection followed by recurrent selection is regularly employed for the breeding of synthetic varieties in bulb onion. Other types of recurrent selection include S_2 family selection, half-sib selection and full-sib selection.

The following steps are important for the development of a synthetic onion cultivar on the basis of S_1 family selection.

1. First year: Selfing of breeding material by bagging.
2. Second year: Evaluation and selection of good bulbs between and/or within S_1 lines.

3. Third year: Intercrossing among good S_1 plants.
4. Fourth year: Sowing of the obtained seeds and selection of good plants.
5. Subsequent years: Use of bulked seeds as synthetic cultivars after four to five cycles of S_1 family selection.

Because recurrent selection takes a long time, it is not frequently used for the breeding of outcrossing crops. Onion breeders often give up on recurrent selection after only a few cycles because of bad results (K. Tsutsui, pers. comm.). However, bulb quality could improve considerably after four to five cycles of recurrent selection, including S_1 family selection (K. Tsutsui, pers.comm.). To illustrate synthetic onion breeding the development of Japanese synthetic varieties is presented in Figure 3.

The onion introduced to Japan at the end of the nineteenth century was not suitable for the Japanese climate and adequate pesticides did not exist at that time. Under such harsh environmental circumstances, Japanese farmers developed several unique OP varieties. These old Japanese varieties were obtained through synthetic breeding. The development of such a variety is difficult even if a breeding technique is backed-up with modern cultivation techniques. The onion varieties introduced at the end of the nineteenth century have a rich diversity of favourable genes, and modern onion breeders have not improved on them by using recurrent selection. In fact, even breeders of corn, who commonly use recurrent selection, tend not to recognize its importance (Hallauer, 1992).

7.3 F_1 Hybrid Breeding

The practical aspects of F_1 hybrid breeding has been comprehensively treated in a number of publications to which the reader is referred to, namely Dowker and Gordon (1983), Pike (1986), Kaul (1988), and Dowker (1990). An overview of the development of F_1 hybrid variety development is shown in Figure 4. The development of F_1 hybrid cultivars in onions can be described as follows:

1. First year: Selfing of breeding material by bagging. The female tester is enclosed in the same bag (Figure 4). Generally, blowflies or bees are used for cross-pollination.
2. Second year: Evaluation and selection of good bulbs among S_1 families and/or within each S_1 family as well as among the corresponding F_1 families (Figure 5).
3. Third year: Checking for male fertility in each F_1 family. If all plants of an F_1 family are sterile, the corresponding S_1 family can be used as a maintainer. For BC_1 production, the F_1 is crossed with a maintainer in a small cage (Figure 6). The S_1 family which is not a maintainer is used to increase the production of S_1 seeds and to produce F_1 seeds in another small cage. At the same time, the S_1 and F_1 seeds obtained the previous year are sown, and then the S_1 and F_1 bulbs are revaluated. The cage mass for the S_1 family discarded by re-evaluation should be discontinued. If needed, an S_2 family is obtained via the selfing of S_1 plants.

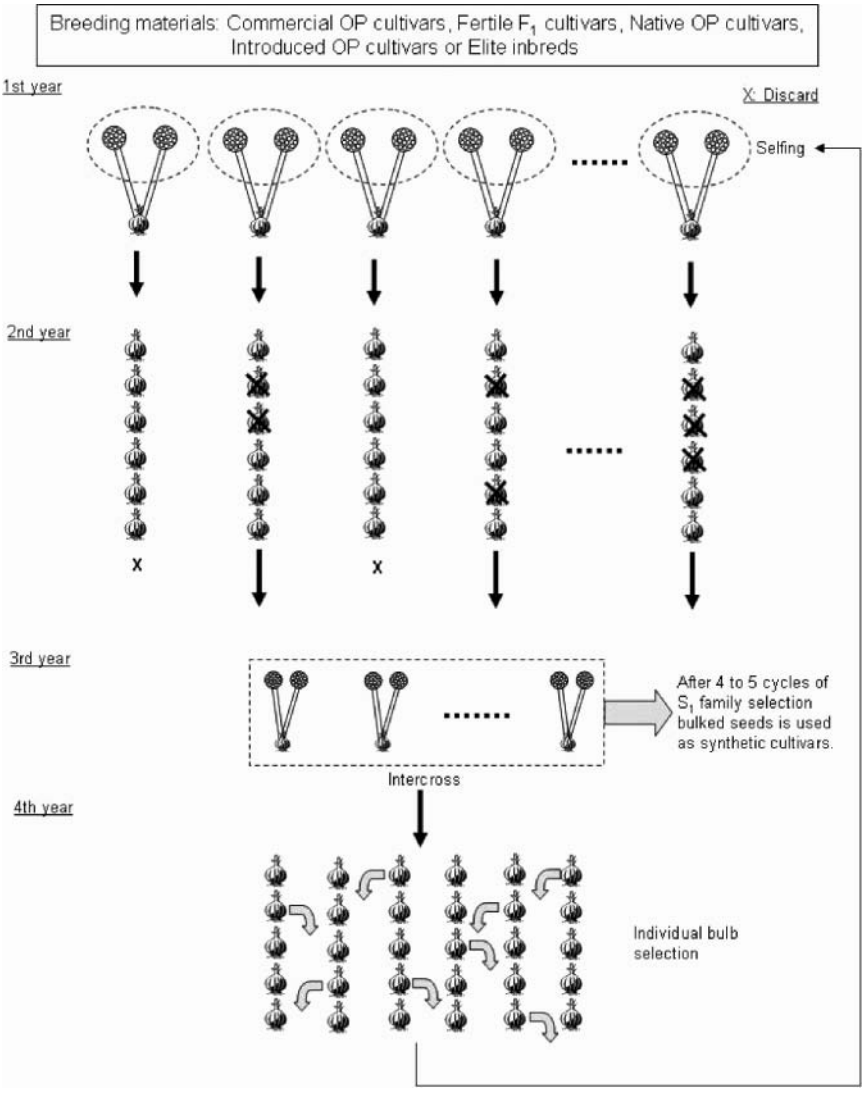
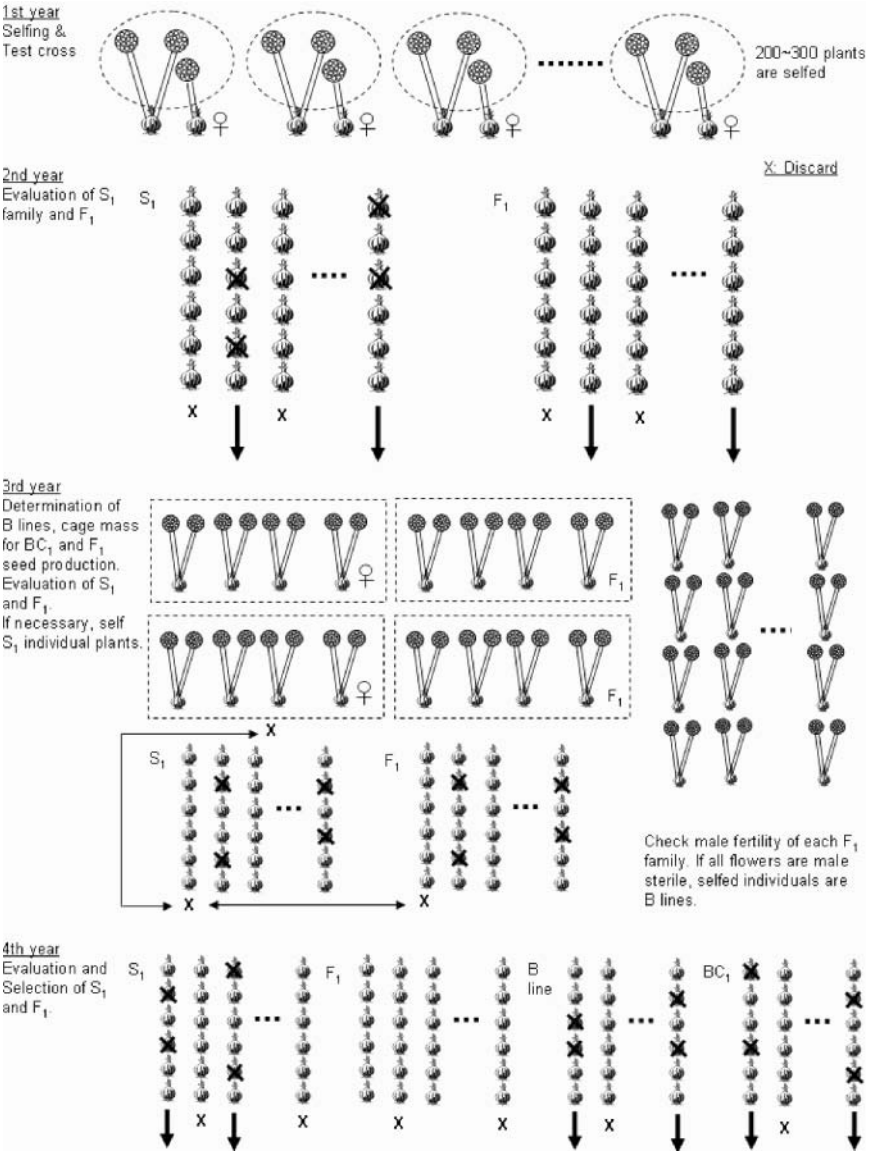


Fig. 3. General breeding scheme for the development of a new synthetic variety of onion via S_1 family selection.


1st year
Selfing &
Test cross
200~300 plants
are selfed
2nd year
Evaluation of S1
family and F1
X: Discard
S1
F1
3rd year
Determination of
B lines, cage mass
for BC1 and F1
seed production.
Evaluation of S1
and F1.
If necessary, self
S1 individual plants.
Check male fertility of each F1
family. If all flowers are male
sterile, selfed individuals are
B lines.
4th year
Evaluation and
Selection of S1
and F1.
B
line
BC1

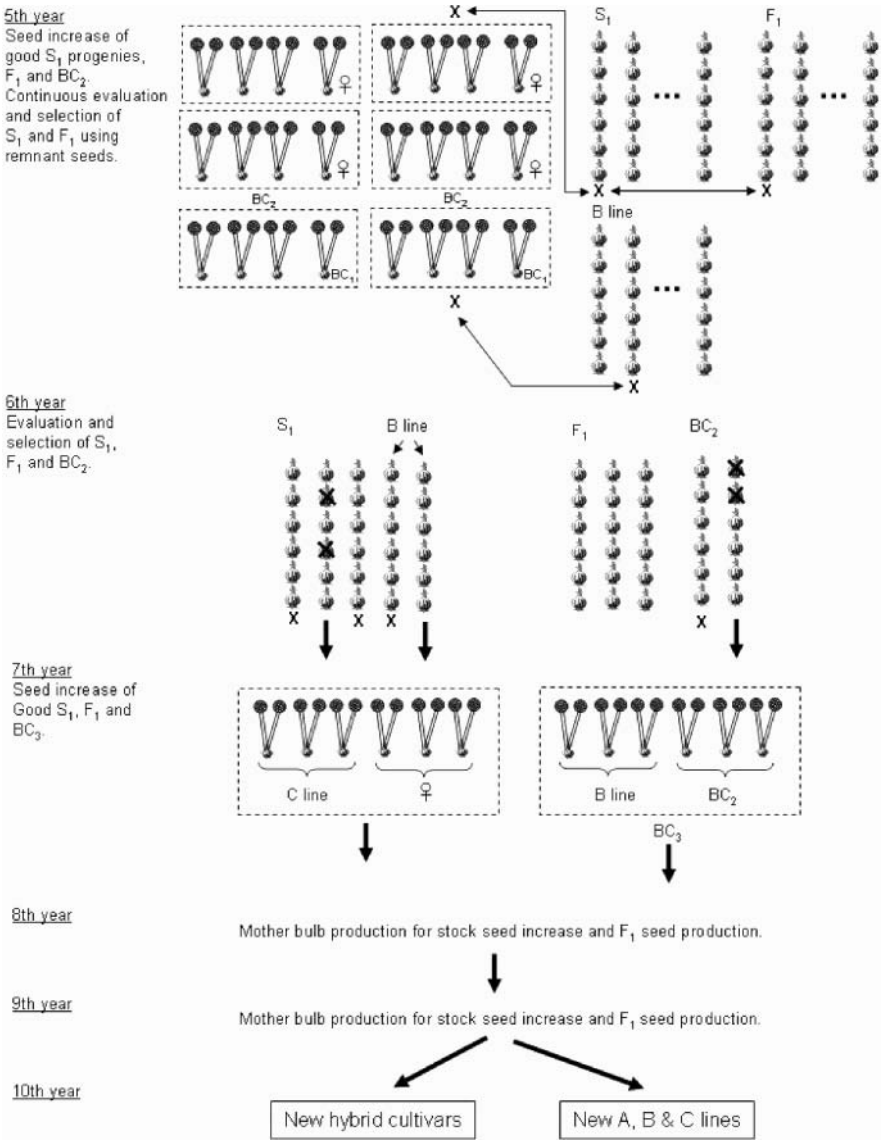


Fig. 4 (continued). Summary of a general breeding scheme for the breeding of new onion F_1 hybrid varieties.

Fig. 5. Small bags are used to obtain S_1 and F_1 families at the same time.

Fig. 6. Small cages are used to cross F_1 plants with maintainers in order to obtain BC_1 families.

Fig. 7. Mother bulb storage in an open, well-ventilated shed in Japan.

Fig. 8. Typical greenhouse seed production for a commercial F_1 cultivar.

4. Fourth year: Sowing of the seeds obtained in the previous year and selection of good S_1 (B and C lines), F_1 and BC_1 plants. Good plants are kept; the others are discarded.

5. Fifth year: Sowing of remaining seeds obtained in the third year and revaluation of S_1 and F_1 plants. Increased production of S_1, F_1 and BC_2 seeds.

6. Sixth year: Final evaluation of S_1 and F_1 plants and selection of elite lines.

7. Seventh year: Increased production of seeds in the elite lines.

8. Eighth year: Mother bulb production for stock seed increase (Figure 7).

9. Ninth year: Commercial F_1 seed production (Figure 8). The increased production of female-line seeds (BC_4) and stock seeds (B line and C line).

10. Tenth year: Establishment of new hybrid cultivars and new A, B and C lines.

In the Netherlands a LLD F_1 hybrid breeding cycle mostly starts with the inbreeding of around 500 parental plants and after two-three inbreeding and selection cycles less than five selfed progenies are entered into the F_1 hybrid testcrossing program.

8 Integration of New Biotechnologies in Breeding Programs

8.1 Genetic Mapping Research

Genetic linkage maps have proven to be in several crops a significant step forward in fundamental genetic studies as well as in practical breeding programs. In onion the first molecular markermap was developed by Havey and co-workers (King et al., 1998). This low-density RFLP map was based on an intraspecific cross between cultivar Brigham Yellow Globe (BYG) and cultivar Ailsa Craig (AC). The mapping population size was 58 genotypes (F_2 derived F_3 lines) and 116 markers were mapped in 12 linkage groups covering 1060 cM. The study showed that the onion genome consists of a large proportion of duplicated sequences and it was suggested that intrachromosomal duplication could be the mechanism behind this duplication. Subsequently Van Heusden et al. (2000a) constructed a low density AFLP map based on a selfed progeny (65 plants) from an interspecific cross between *A. cepa* cv. Jumbo and *A. roylei* C502. In total around 500 markers were mapped on both parental maps. The onion map consisted of eight linkage groups covering 694 cM and the *A. roylei* map of 15 linkage groups covering 624 cM of the expected 800 cM. Using monosomic addition lines van Heusden et al. (2000b) assigned the eight different onion linkage groups to individual chromosomes. Furthermore a substantial number of *A. roylei* linkage groups could be linked to individual chromosomes. On both intra- and interspecific maps only a few agricultural important traits could be mapped amongst which the Ms locus for the S cytoplasm, the downy mildew resistance locus and an alliinase gene.

In the last five years new markers and QTLs have been added to the intraspecific map(s) notably QTLs involved in the organo-sulphur and carbohydrate metabolism. Both traits are of importance in commercial onion programs as they determine pungency (organo-sulphur) and bulb hardiness (carbohydrates). It was shown by Galmarini et al. (2001) that pungency and soluble solids are genetically linked to

each other, which explains the difficulty in commercial onion programs to select for these traits independently. Havey et al. (2004) detailed this picture as they suggested that onion bulbs which accumulated fructans take up or retain less water, which leads to a concentration of both soluble solids and organo-sulphur compounds. McCallum et al. (2001) improved the intraspecific (BYG x AC) onion map by sequencing a large number of cDNA clones which revealed previously RFLPs. This resulted in the deposition of around 128 ESTs to public databases. Furthermore they introduced a new intraspecific mapping population next to the BYG x AC population, namely a population based on a selfing of an individual that originated from a cross between cv. Colossal and cv. Pukekohe Longkeeper. In 2004 Kuhl et al. developed a unique set of around 11,000 EST sequences which will prove to be of great value in future genetic and breeding research. Furthermore they showed that there are large differences between the monocot orders of the Asparagales (*Allium* crops) and the Poales (grasses and cereals), which proves that maps developed for the Poales can not be used for the Asparagales. Recently Martin et al. (2005) linked the BYG x AC linkage groups to individual physical chromosomes using monosomic addition lines which were previously used in the study of van Heusden et al. (2000b), furthermore the BYG x AC map was enriched with another 100 EST based markers.

Interspecific mapping research using a segregating population between onion and the interspecific hybrid between *A. roylei* and *A. fistulosum* resulted in the development of a linkage map of 450 AFLP markers which were distributed over eight linkage groups which were assigned to individual chromosomes (De Melo, 2003). Distorted segregation took place for one-quarter of the markers. On this map the genetic basis of a number of rooting traits were analyzed, as this work was carried out in the context of organic farming, a farming system in which onions experiences more harsh conditions as in conventional cultivation systems especially concerning the uptake of water and nutrients. For a number of rooting traits the genetic basis could be analyzed: for the number of bulbs one QTL was found, for the number of stem-borne roots per plant (two QTLs) and the number of lateral roots per stem-borne root (one QTL). Subsequently Khrustaleva et al. (2005) used the same population for integrated mapping. Integrated mapping, the combined analysis of both recombination and physical maps, has been carried out predominantly in the monocots for cereals and it was found amongst others that genes were predominantly located on the distal ends of chromosomes. However the *Allium* integrated mapping research showed that this might not be the case for this group of species as indications were found that pointed into the direction of genes located on the more proximal end of chromosomes, at least for the studied chromosomes 5 and 8.

Another way to analyze the genetic basis of traits and to assign molecular marker linkage groups to physical chromosomes is via the use of so-called monosomic addition lines (AMALs). A complete set of *Allium fistulosum* – shallot (*A. cepa* Aggregatum group) monosomic addition lines was established by Shigyo et al. (1996). For an overview on the genetic studies involving these *Allium* AMALs the reader is referred to Shigyo (2006). The AMALs proved to be very effective in revealing the effects of single alien chromosomes from *A. cepa* on the production of for example flavonoids and anthocyanins (Shigyo et al., 1997) and carbohydrates (Hang et al., 2004) in the leaf tissue of *A. fistulosum*. Furthermore the assignment of

all structural genes involved in flavonoid biosynthesis influencing bulb colour to individual chromosomes could be accomplished by direct comparisons between the chromosomal constitution and the flavonoid contents of the scaly leaves in *A. fistulosum* – shallot multiple addition lines (Masuzaki et al., 2006a, 2006b). Also the AMALs played and important role in the linkage of several intra- and interspecific molecular marker maps to physical chromosomes (Van Heusden et al., 2000b; Martin et al., 2005).

Molecular marker mapping also takes place in bunching onion (*Allium fistulosum*) as this crop, which is a close relative to onion, is of high economic importance in Asian countries (Inden and Asahira, 1990). The first map that was developed for bunching onion was an AFLP map and this map was based on backcrosses between two partially inbred lines (S_2) from the Senju (cv. Saiko) and Kujo (cv. Kujo Futo) group (Ohara et al., 2005). In total two maps were constructed, on the first 164 markers could be mapped on 15 linkage groups and on the second 120 markers were mapped on 14 linkage groups. Nine linkage groups from both maps could be connected to each other. Recently Tsukazaki et al. (2006) reported on the development of an SSR map for bunching onion based on 193 markers which were located on 15 linkage groups and covered a map distance of around 1500 cM. The map was based on an F_2 population originating from a cross between cv. Saiko and cv. Kujo Futo. Future genetic research on bunching onion will address the mapping of disease and pest resistances on this map and quality traits like pungency and sugar content. Also developmental traits like late bolting and seedling growth will be investigated. Furthermore the chromosomal assignment of these two linkage maps is currently taking place via the use of the shallot - *A. fistulosum* alien addition lines developed by Hang et al. (2004b).

Future mapping research will onion and its crossable relatives certainly needs to integrate all the efforts made so far to produce a truly integrated map for all eight physical chromosomes. In this way fundamental and breeding genetic research can benefit too a large extent from all the work that has been done until present.

8.2 Doubled Haploids

Inbreeding in onion is problematic and this is the reason why inbred lines used for the development for F_1 hybrids are in most cases only two-three times selfed. However such lines will not have a high degree of genetic and phenotypic uniformity. Therefore present onion F_1 hybrids, which are based on the crossing of two partially inbred lines, show considerable phenotypic variation. To circumvent this problem doubled haploid (DH) technology might provide a way forward, as DH lines can be obtained in a short time and will possess a high genetic uniformity. It has been shown in the past that haploid induction in onion is possible (Muren, 1989; Keller, 1990; Campion et al., 1992; Martinez et al., 1997). However DH in onions is based on gynogenic embryo induction and not on the more efficient technique of microspore culture. As explant material in onion DH production, flower buds are used and haploid induction frequencies proved to vary to a large extent. In a study of Bohanec and Jakse (1999) induction frequencies between the 0 - 3.7% (22.6 embryos per 100 cultured flowers) were reported. These frequencies depended strongly upon

the genotype used. Therefore the genetic basis of this trait should be studied into more detail. Genome doubling is a further problem in this context as only 10% of the embryos develop into diploid plants. Jakse et al. (2003) showed that the usage of amiprofos-methyl (APM) might be beneficial as this compound resulted in the production of ca. 25% diploid plants with a limited reduction of the survival (around 24%) of the embryo's. Luckily genetic instability of the regenerants proved to be not an important phenomenon (Campion et al., 1995; Javornik et al., 1998), however the fertility of the DH lines was variable. All in all, the current limiting factors in the application of DH technology in onion breeding are the genotypic dependency of induction frequency and the severe inbreeding depression. One way to overcome the genotypic dependency is the crossing of high inducing lines with lines elite lines with lower induction frequencies. To overcome the inbreeding depression and its effect on fertility is more difficult, however the induction of DH lines itself is a strong selection factor to reduce this problem and repeated hybridization of DH lines and selection of the most fertile genotypes would be an added factor.

8.3 Transgenic Plants

For a long time, onions proved to be recalcitrant towards genetic transformation. Klein et al. (1987) were the first to prove that onions could be transformed using a high-velocity microprojectile method. A decade later Wang (1996) produced transgenic leek plants (*Allium porrum*) via particle bombardment. For garlic, Barandiaran et al. (1998) and Ferrer et al. (2000) showed that it is possible to transform garlic by means of particle bombardment. However all the aforementioned work carried out in the previous century resulted in transient expression of the (reporter) genes transformed, so no genetically stable transgenics were produced.

Dommisse et al. (1990) proved that onion is a host for *Agrobacterium tumefaciens* and it was this important finding that led in 2000 via the work of Eady and coworkers to the development of genetically stable onion transformants. Zheng et al. (2001) improved the transformation genetically stable transformants of onions and shallots using mature instead of immature embryos and via this method it is currently possible to produce transgenic onions whole year round. Kondo et al. (2000) were the first to develop stably transformed garlic also using *Agrobacterium* as a vector. Zheng et al. (2004) developed an efficient *Agrobacterium* transformation protocol for garlic using calli from root segments both apical and non-apical. All current *Agrobacterium* methods have reported transformation frequencies around 2%, therefore genetic transformation of *Allium* crops is still a matter of large numbers.

Nowadays transgenic onions have been developed carrying herbicide resistance (Eady et al., 2003). The herbicide resistance is based on the Basta gene making onions resistant to Round-up. This herbicide resistance trait is very useful in onion cultivations worldwide as onions are known to be weak competitors to their accompanying weeds in cultivations. Zheng et al. (2005) developed tropical shallots resistant to beet armyworm (*Spodoptera exigua*). The resistance to beet armyworm is based on a Cry gene. This trait is important in tropical onion and shallot cultivations as considerable losses can occur in cultivations due to this pest. Although both

herbicide and beet armyworm resistant onions and shallot transgenics have been developed, no cultivars based on these genes are present on the world seed market. This is amongst others due to the high costs involved for the registration of these transgenics cultivars (estimated around 10 million € per cultivar), the requirement to separate the transgenic and non-transgenic seed production at breeding companies and further down the chain and the negative public opinion in various countries towards genetic modification. Therefore it will probably take still some time for the introduction of GMO onion cultivars to the seed market.

8.4 Interspecific Hybridization

Although interspecific hybridization is not a 'new biotechnology' it can substantially improve the breeding of new cultivars. Until recently not much use has been made in onion breeding to exploit wild crossable relatives. The only exception in this context is *Allium fistulosum*, which is actually not a wild species but a domesticated species which originates from *A. altaicum*. *A. fistulosum* is an interesting gene reservoir as it carries a number of traits which are of agronomic importance for onion like, earliness, resistance to onion leaf blight and pink root, high dry matter content, winter-hardiness, etc (Rabinowitch, 1998). However introgression of *A. fistulosum* into onion proved to be problematic (Van der Meer and Van Bennekom, 1978) and only some minor varieties were developed like the amphidiploid 'Beltsville Bunching' and the triploid hybrid 'Delta Giant', both of which are still cultivated in the USA. The real interest in the exploitation of wild relatives for the breeding of new onion cultivars came in the late eighties of the previous century when its was found that *A. roylei* was completely resistant to downy mildew and had a high partial resistance to onion leaf blight (for review see Kik, 2002 and section 5.2.1 of this chapter). A further example of a successful introgression into the onion germplasm is the introduction of the (or a) cytoplasm of *A. galanthum* in the nuclear background of onion, which proved to give cytoplasmic male sterility (Yamashita and Tashiro, 1999; see also section 5.1.2 of this chapter). Interestingly Khrustaleva and Kik (2000) showed that it is actually possible to exploit *A. fistulosum* for onion breeding when *A. roylei* is used as a bridging species between these two species and this opens interesting perspectives for the breeding of new onion cultivars which have improved resistances against a number of diseases, like downy mildew, onion leaf blight, but also Fusarium basal rot, pink root and anthracnose. Furthermore *A. fistulosum* has a much better rooting system then onion and this can be of value in the breeding of onion cultivars for more harsh conditions like in the case of organic agriculture. In this context De Melo (2003) showed in the cross between onion and the interspecific hybrid between *A. roylei* and *A. fistulosum* that the genetic basis of a number of rooting traits had only a few major QTLs which should make the introgression of these valuable traits possible into the genetic background of onion.

9 Seed Production

There are several issues that need to be considered in onion seed production:

- The bulb-to-seed method, which allows the grower to discard undesirable bulbs, is suitable to ensure consistency in the shape and size of F_1 hybrid onions, whereas the seed-to-seed method, which does not require bulb production, is more suitable for growers who wish to reduce the time invested in onion production.
- A three-way-cross hybrid with the genotype of (A x B) x B or (A x B) x C is useful for increasing the seed productivity of the female parent, but often leads to less consistency in shape and size. For this reason, unless there is a need to boost the seed production level of the female parent, a single-cross hybrid (i.e., A x B, A x C) is a more desirable way of producing hybrid seeds.
- Flowering time and duration of the male parent should match those of the female parent.
- Honey bees are used for pollination. The use of insecticides immediately before or during the flowering season must be avoided.
- High potassium levels and the pungent smell of onion nectar make onion flowers less attractive to honey bees. Even with ideal weather for pollination, the presence of bee-attracting flowers in the vicinity of the seed production field during the flowering season may lead to a reduced seed yield.
- The purity of the female parent should be maintained by carefully preventing any fertile male strains from getting mixed into the female plant lines.
- The seed production fields of different varieties should be separated from each other by the linear distance of at least 4 kilometres to avoid the risk of unintended cross breeding.
- The male-female ratio in the seed production field should be within the range of 1:2 to 1:4, depending on the reproductive capacity of the male parent.
- Too much rain during the flowering season may interrupt bee pollination and decrease the amount of pollen released in the atmosphere, leading to a poor seed yield.
- Hot and arid climate during the flowering season may also lead to a reduced seed yield because bees may stop visiting flowers in search of water for cooling down their hives. Furthermore, such weather may lead to a high incidence of thrips, which could cause serious yield loss.
- In the cultivation areas of the Hokkaido prefecture in Japan, most of the onion growers raise their onion seedlings in cell trays before being planted (Figure 9). This is why onion breeders have to prepare high quality seeds with more than 95% germination rate (Figure 10).

In grains and beans, the edible portion of the plant can be both used for consumption and kept to be used as seeds. Therefore, any improvement made on the edible portion automatically enhances the seed quality. On the other hand, the seed and edible portions of the onion plant (i.e., the bulbs) are not the same, and although some improvement and upgrading have been made on the bulbs, little or no effort has been made to improve the quality of seeds. One of the most important aspects of seed improvement is, of course, enhanced seed yield. In this respect, relatively little attention has been given to pollinators, carriers of pollen from the male parts of a flower to the female parts of another flower. However pollinators are very important and in some years, there is a sharp decrease in the number of honey bees visiting

onion flowers. The reason behind this and the method to increase bee visits are yet to be discovered.

Fig. 9. A machine for transplanting onion seedlings.

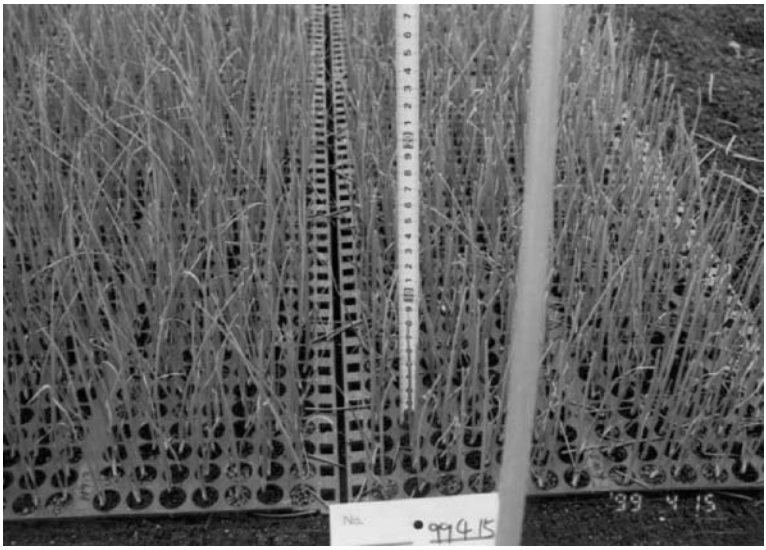


Fig. 10. Onion plants raised in cell trays.

10 Conclusions and Future Directions

Since the reviews of Pike (1986) and Dowker (1990) on onion genetics and breeding significant steps forward have been made in this research area.

On the breeding level, onion breeding has changed significantly in the last twenty years as the overriding majority of the cultivars which appear on the seed market nowadays are F_1 hybrids. In this context, one wonders if the use of only two CMS sources does not make the crop vulnerable in a similar way as CMS Texas made maize vulnerable for Southern corn blight. The development of new CMS sources like CMS-galanthum might provide a way forward in this respect. Next to yield, skin retention and sprouting dormancy in temperate onion breeding, also other traits have become more important, like disease resistance and mild taste. Especially in the area of disease resistance breeding significant steps forward were made (downy mildew, FBR). However still major disease problems in onion cultivation exist for example white rot (*Sclerotium cepivorum*) and purple blotch (caused by *Alternaria porri*) which both give worldwide large problems every year. Also pests like thrips (caused by *Thrips tabaci*) and onion maggot (*Delia antiqua*) result annually in significant crop losses. Other traits which became increasingly important in the last years are bulb-colour and shape as, like in other vegetable crops, also in onions the trend is from quantity to quality and this is especially true for the Western markets. Furthermore new niche markets for onions are emerging such as the organic market. On a global scale it will be of interest to see if and how the onion market will change as large onion producers like China and India will enter the international trade market.

On the research level genetic linkage maps covering the whole genome have been developed in the last two decades using molecular marker technology. On these maps QTLs for quantitative traits like pungency and dry matter content were identified and also markers closely linked to the resistance gene for downy mildew and the restorer gene for the CMS-S cytoplasm have been located on linkage maps. The next challenge in the area of onion linkage mapping is the development of a unified *Allium* linkage map which not only relates the current intraspecific onion linkage maps with each other but also the existing interspecific maps with species like *A. roylei* and *A. fistulosum*. The presence of (monosomic) addition lines will certainly be of great help in accomplishing this work. Also the emerging research on onion genomics will certainly be very instrumental in this respect and via this type of research it will also be very fascinating to see in future how for example the huge onion genome is being build-up. Increased insight has also been gained in the last two decades on onion CMS: the existence of various cytoplasms, the distribution of these cytoplasms and their restorers over worldwide onion populations, the putative origin of these cytoplasms, the differences in micosporogenesis between onion cytoplasms, etc. Also in this case markers were developed which aid breeders nowadays in identifying CMS and normal cytoplasms. The renewed interest in interspecific hybridization in onions has also been of importance in the last two decades, not only from a breeding point of view but also for fundamental research in the area of genome organization. Especially the possibility for the application of GISH technology in onion introgression research gave much insight in the genome

composition of complex hybrids. In genetic transformation also significant steps forward have been made as in the eighties of the previous century no *Allium* crop could be genetically modified. Nowadays genetic modification can be routinely applied in the development of onion GMO's and herbicide and Bt onion transgenics have already been developed and are waiting to be released on the market. Doubled haploid (DH) technology has also been developed for onion. The technique is not based on microsporogenesis but on gynogenesis making the production of large numbers of (doubled) haploid plants less practical. Furthermore, the finding that onion heterosis is not based on overdominance but rather on partially dominant alleles makes that DH technology in hybrid breeding is less obvious. However the increase in uniformity, the ease of introgressing traits and the easy handling of DH lines in the hybrid breeding process makes this DH technology still an interesting option for onion breeding. Last but not least, onion genetic resources have also received considerable attention in the last two decades and international databases have been created in which onion genetic resources can be found. An important goal for future onion genetic resources will be the development of a global *Allium* virtual genebank, which is well characterized and evaluated, and cleared from duplicated accessions. Next to this an equally important goal is conservation of *Allium* genetic resources via collection missions or *in situ* conservation activities, as it has been observed a number of times that genetic erosion is taking place rapidly.

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Eggplant

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1 Introduction

Solanum melongena L. (known as eggplant in the United States and aubergine in France and England) is one of the few cultivated solanaceous species originating from the Old World. It is known as brinjal in its home country, India, where it was domesticated long ago and where the greatest diversity is found. Widely grown in temperate and tropical Asian countries, eggplant has been also a common crop in the Middle East and around the Mediterranean basin and is now cultivated worldwide. The ethno-botanical history of eggplant is quite fascinating given its uses as food crop, medicine, and ornamental by Ancient (Indian) and Medieval (Arabic and European) civilizations, and the diverse beliefs surrounding its use including aphrodisical properties and various malevolent and benevolent effects. In the beginning of the 21st century, the health values of eggplant are being revived once again based on phenolic and alkaloid content.

Eggplant belongs to the very large genus *Solanum*, as well as its largest subgenus, *Leptostemonum*, which includes many wild relatives, as well as other cultivated species, such as the Gboma eggplant (*Solanum macrocarpon* L.) and the scarlet eggplant (*Solanum aethiopicum* L.) grown mostly in Africa for their fruits and leaves. We will focus here on *S. melongena* (for a review of African eggplants, see Daunay et al., 2001a). The taxonomy of eggplant relatives still remains a challenge. The continuum of morphological variation, cross compatibility, and genetic distances which exist between advanced and primitive cultivars of eggplant, with weedy and wild forms and relatives provides a model system for the study of gene flow of traits affected by domestication between a crop and its spontaneous forms.

Although an Old World taxon, *S. melongena* unexpectedly shares strong genetic similarities with New World *Solanum* species (tomato and potato). These syntenic features have been only recently discovered and will undoubtedly assist in an

understanding of the genes controlling eggplant key horticultural traits. Biotechnological techniques underway with eggplant and its relatives offer new tools to complement conventional breeding strategies for genetic improvement for the benefit of growers and consumers. In this review, we will guide the reader through a network of botanical, historical, economic, biological, and genetic information which is intended at being useful to future eggplant breeders.

2 Origin and Domestication

Based on morphometrics, crossability, seed coat scanning electron microscopy, leaf isozymes, and seed protein electrophoresis studied on a large set of accessions, Lester and Hasan (1991) assumed that ancestral forms of *S. melongena* originated from African tropics (where many wild relatives of eggplant are still found) and the Middle East. Indeed one finds in these areas the closest wild relatives of *S. melongena* which belong to the *S. incanum* aggregate (Lester and Hasan, 1991; Daunay et al., 2001a). This latter is a quite complex group. One can find an example of the difficulty to typify it in Lester (1997). *S. incanum* agg. brings together four species (or groups), named *S. campylacathum* (group A) distributed across the East African tropics, *S. delagoense* (group B) in South-Eastern Africa, *S. incanum sensu stricto* (group C) in North-Eastern Africa and Middle East, and *S. lichtensteinii* (group D) in South-East Africa. Lester and Hasan (1991) developed the scenario that representatives of *S. incanum* agg. migrated eastwards, either spontaneously or as a result of human migrations during pre-historic times. Indeed, the roots, leaves and fruits of these species are variously used for instance for medicinal purposes, leather tanning and milk curdling (Bukenya and Carasco, 1999; Lester and Hawkes, 2001). According to Lester and Hawkes (2001), the fruits are not eaten. However, Amar (2000) reports the use as food of the fruits of a local wild semi-perennial eggplant (possibly *S. incanum* Group C) in the lower Jordan valley at the time of the Mamelouk period (1250-1517).

Deb (1989) reported that *S. incanum* is found as a wild plant in Southern India, but Lester and Hasan (1991), based on a wide experimental and analytical research, ascertained that the Indian putative "*S. incanum*" is a wild form of *S. melongena*. *S. incanum* differentiated progressively in South East Asia into a closely related species, the wild *S. melongena*, which is still found growing spontaneously in a large area from Southern and Eastern India to Southern China, Philippines and Indonesia, and is described by former botanists as *S. cumingii*. Subjected to a progressive domestication process, this wild form gave rise to primitive eggplant cultivars known as *S. ovigerum*, with small round or oblong fruits, white, green or violet, which evolved progressively into advanced cultivars with large sized fruits. *S. insanum*, widespread in India, is probably a form of *S. ovigerum* which reversed to the wild state, developing a strong prickliness (character favourable for escaping animals grazing), a low straggling growth habit and a brief life cycle. Lester and Hasan (1991) bring all these taxa under the umbrella of *S. melongena,* structured as group E (*S. insanum*), group F (*S. cumingii*), group G (*S. ovigerum*) and group H (advanced cultivars). The case of a Thai cultivar type with vigorous plants setting round green

ping pong sized berries is interesting because it is considered there as an advanced cultivar type, although the size (and colour) of its berries are much closer to those of the wild *S. incanum* than to those of a classic advanced cultivar, characterized by much larger fruits. This cultivar type can be interpreted as a cultivate survivor of an early domesticated form.

The proposed scenario of *S. melongena* origin and domestication seems appropriate based on available data which demonstrate a pseudo-continuum of morphological features, cross compatibility, and genetic distances between groups A, B, C, D of *S. incanum* and the groups E, F, G, H of *S. melongena*. This scenario is open to refinements, anytime new data will bring new insights on *S. melongena* evolution history.

Later taxonomic studies were based (1) on diverse combinations of accessions belonging to groups A, B, C, D, E, F, G or H, together with other *Solanum* species, and (2) on the use of allozymes (Isshiki et al., 1994a; Karihaloo and Gottlieb, 1995), enzymes (Kaur et al., 2004), seed proteins (Karihaloo et al., 2002), chloroplastic DNA (Sakata et al., 1991; Sakata, 1992; Sakata and Lester, 1994 and 1997) and nuclear DNA (Karihaloo et al., 1995). All these studies converge for confirming the close relationships between the A to H groups, and demonstrate the large diversity in the *S. incanum* group and the narrow variability with the *S. melongena* group, thus confirming a genetic bottleneck event during eggplant evolutionary history and domestication from a narrow gene pool (Lester and Hasan, 1991). Using the highly polymorphic AFLP, Mace et al. (1999) confirmed the separation between groups A, B, C, D, E, F, G and H, and revealed some variation within groups C (*S. incanum* sensu stricto) and group E (weedy form of *S. melongena*).

The classification work carried out by Lester and Hasan in 1991 found a brilliant confirmation in the recent phylogenetic analysis of the *S. incanum*/*S. melongena* species complex, based on both chloroplastic and nuclear DNA sequence data (Weese and Bohs, 2006).

3 Crop History

The first records of the use of *S. melongena* are found in Sanskrit documents, which are dated in the beginning of the Christian era (Bhaduri, 1951; Khan, 1979a and b). The extraordinarily large number of words for the eggplant in Sanskrit and the fact that some of them are not only descriptive but also highly complimentary suggest that the eggplant was not only common but was also popular in the ancient days with Sanskrit-speaking people. The mention of eggplant is also found in Chinese botanical and agricultural treatises of about the same period such as the 'Atlas of plants in Southern China' written during the Western Jin Dynasty (AD 265 ~316), the 'Qimi Yiaoshu' a practical handbook of agriculture written at the time of the Southern and Northern Dynasties (AD 420~581) (Xu Z., pers. commun.) and in the Ts'i Min Yao Shu, a Chinese work on Agriculture of the fifth century (Bretschneider 1882, quoted by Hedrick, 1919). From China, eggplant reached Japan about the 8th century at the time of the Tung dynasty (Allard, 1996) where it became a common vegetable. When eggplant reached Afghanistan and Persia is still unclear. Further

research in historical and archaeological documents would undoubtedly bring further insight to the spread of the species throughout Asia and Middle East.

According to the Encyclopedia Iranica (1988), eggplant was brought to the Iranian lands at a very early but indeterminable date, and Iranian and Arab sailors carried it to East Africa in ancient times as shown by the presence of many specific terms for it in Ethiopia. The same source indicates that the Medieval Iranian writers on medicine and botany urged caution in use of the eggplant, but mentioned also its culinary use. Eggplant reached the Eastern Mediterranean lands probably after the Arab conquest of Iran. The conquering Turks got to know the plant in Iran. Eggplant was known neither by Greeks nor by Romans. Its spread through the Mediterranean Basin is linked to the Muslim conquests from 8th century onwards. According to Lobel (1581) and de Candolle (1883), the Persian physicians Rhazes (864-925 AD) and Avicenna (also known as Ibn Sina, 980-1037) mentionned eggplant, as well as the Andalusian-Arab physician Averroes (also known as Ibn Rushd or Abu al Walid, 1126-1198). The Moorish Spaniard Ibn Al Awam (12th century) describes in his book on agriculture the techniques recommended for growing eggplants, and he mentions the presence of the crop in Syria, Egypt and Sicily. According to de Candolle (1883) the physician Ebn Baitair who lived also in Spain in the 13th century mentioned eggplant, and Hedrick (1919) reports that the German Albertus Magnus (1193-1280) wrote about eggplant. All these historical traces demonstrate the ancient presence of eggplant as medicinal and food crop in Middle East as well as in Europe. The spread of eggplant to Balkan countries is probably linked to the Ottoman Western conquests (14th to 16th centuries).

In the European Medieval medico-botanical treatises (herbals), the eggplant illustrations display medium to large sized fruits, pear shaped to globose, and most often purple coloured (Daunay et al., 2007). In the Renaissance herbals of the 16th century, these fruit types are still present, together with illustrations deriving from the woodcut by Fuchs (1543), displaying plants bearing egg-shaped fruits (Daunay et al., 2007). Long fruited types appear in the herbal by Dalechamps (1586). The texts attached to European Medieval and Renaissance illustrations (paintings or woodcuts) usually mention the noxious effects of eggplant on health. This is due to the association of eggplant to mandrake, and hence to its disquieting properties, probably because of the similarities between the round gold berries of mandrake with those of some eggplant types. This is attested by the Latin name *Mala insana* which according to Dalechamps (1586) was first attributed end of the 15th century by Hermolaus Barbarus (1454-1493) and then widely used by later herbalists. As mandrake, eggplant was also attributed aphrodisiac effects that were alluded to in illustrations. However Renaissance authors conceded also that eggplant fruit was pleasant to eat, and by the beginning of the 17th century, eggplant was a quite common vegetable in Southern Europe.

According to Lobel (1581), Avicenna and Averrhoes were the first to name the plant 'Melongena', derivative of which such as 'Melongiana' and 'Melonge' are found in Medieval herbals of the 14th century, and which was to be used again by Linnaeus in the 18th century in the binomial *Solanum melongena*. There is a wealth of eggplant common names across the Asian, Middle East and Mediterranean countries. They can be gathered into three main groups, having a relationship either

(1) with the negative beliefs surrounding the plant (*Mala insana* is related to 'melanzana' in Italian, and 'melitzane' in Greek), or (2) with the egg shape and size of some cultivars which are at the origin of 'eggplant' in English, and 'Eierfruit' in German. 'Eggplant' suggests that a number of early introductions into Europe had small ovoid white fruit and this is partially confirmed by the study of the iconography (Daunay et al., 2007). The third group is composed of a great number of names deriving from each other in various combinations and which are based on transliterations from Sanscrit, Persian, Arabic and Turkish, and later on from European languages. This group is extremely difficult to unravel without the help of linguists. Examples from De Candolle (1883), Hedrick (1919), Bhaduri (1951), and Khan (1979b) include 'vartta', 'varttaka','vaatingan' or 'bhantaaki' in Sanskrit and 'badanjan', 'bungan' in Hindustani, which are possibly at the origin of 'baadangan', 'baatangaan', 'badenjan' in Persian; which could themselves have derived in 'bedengiam', 'bedengaim', 'badindjan', 'baadanjaan', 'melongena' in Arabic; 'patlidjan' or 'patlican' in Turkish, badnjan in Georgian, 'Tabendjalts' in Berber (North Africa), 'berenjena' in Spanish, 'beringela' in Portuguese, 'bérengène' and 'aubergine' in French. 'Brinjal', one of the common eggplant names in India, derives from the Portuguese *beringela* coined when the Portuguese were the masters of the trade between India and Europe during the 16th and 17th centuries. It is probably at the origin of the French 'bringelle' used in La Réunion Island where people of Indian origin used to live. The complexity of the linguistic study of eggplant names is illustrated by Arveiller (1969), who discusses this topic only for French names throughout 20 pages (!).

4 Socio-Economics and Production

4.1 Economic Data

Production data are provided in Table 1. The world production of eggplant was estimated as 31 millions tonnes (t) (FAO, 2004): China (16,5 millions) and India (8,2 millions) are by far ahead of Egypt (1,1 million), Turkey (900 000 tonnes), Japan (400 000) and Italy 370 000). The yield average is 17 t per hectare, but this figure conceals a great heterogeneity among countries: 18,3 t/ha in China, 34 t/ha in Japan and outstandingly 461 t/ha by very intensive cultural methods of The Netherlands. In Europe, eggplants are mostly grown in southern countries. The production of Turkey, far ahead of other North Mediterranean countries, is mostly consumed locally. Spain (Almeria) and Italy (Sicily) specialized in the last 20 years for exportation to Northern Europe, mostly during autumn, winter and early spring. Due to increasing labour costs, production of eggplant, as for other vegetables, is progressively shifting further south, to Morocco. Due to the partial overlap of greenhouse and open field production of the local and imported crops, eggplant is available to European consumers all year long.

Table 1. Economic data for eggplant (FAO, 2004).

Countries	metric tonnes	Area harvested (ha)	Yield (tonnes/ha)
China	16 530 287	901 572	18.3
India	8 200 000	510 000	16.1
Egypt	1 046 742	43 151	24.3
Turquie	900 000	35 000	25.7
Japan	390 200	11 700	33.4
Italy	366 461	12 379	29.6
Indonesia	312 354	45 285	6.9
Sudan	230 000	12 000	19.2
Spain	175 534	3 892	45.1
Greece	72 547	3 000	24.2
(The Netherlands)	41 000	89	461.7

4.2 Production Methods, Grafting

Eggplant is traditionally grown in open field, but large scale culture in heated or unheated glass and plastic houses developed from the 1970s onwards in all of Europe. Grafting is mostly used in conditions of intensive production. In Europe, eggplant is grafted mostly onto tomato or tomato interspecific hybrids (*L. esculentum* x *L. hirsutum*) which, in addition to their resistance to several soil born pathogens, have a good tolerance to low soil temperatures, thus allowing an early yield in winter and spring production (Ginoux and Laterrot, 1991). *Solanum torvum*, a wild species which has also a wide range of soil borne disease resistances (see section 6), is another valuable rootstock which brings also a higher yield, but its use is limited so far by the difficulty to get a rapid and homogeneous germination (Ginoux and Laterrot, 1991). Though *S. torvum* is resistant to *Verticillium* wilt (see section 6.3), cases of wilt in commercial culture of grafted eggplant have been reported (Garibaldi et al., 2005). Bletsos et al. (2003) obtained positive effects on eggplant scion growth, fruit production and control of *Verticillium* wilt by using *S. torvum*, and obtained similar results with *S. sisymbriifolium* though the control of *Verticillium* wilt was less efficient.

Research on rootstocks for eggplant is the most active in Asia, in particular Japan and Korea, where grafting technique was used for vegetables as soon as the 1920s (Lee, 1994). The first type of rootstocks is *S. melongena* lines and hybrids that resist to *Fusarium* and bacterial wilt (Monma et al., 1997; Yoshida et al., 2004 a and b). The control of *Phomopsis* blight as well as windfall resistance is also reported by Gao MeiXiu et al. (2001), as well as variation of fruit epidermis and flesh firmness (Suzuki et al., 2004) when using eggplant rootstocks.

The second type of rootstocks are based on the use of *S. integrifolium* - i.e. *S. aethiopicum* Aculeatum Group - which resists to *Fusarium* and bacterial wilts, and presents a good graft affinity with *S. melongena*. It is used directly as a rootstock (Mochizuki and Yamakawa, 1979a; Yoshida et al., 2004b) or as parent crossed with *S. melongena* varieties for producing interspecific hybrid rootstocks [*S. integrifolium* x *S. melongena*] cumulating resistances from both parents (Sakai, 1984; Mian et al., 1995).

The third type of rootstocks is other *Solanum* species. According to Yoshida et al. (2004a) *S. torvum* and *S. sanitswongsei* -i.e. *S. kurzii* - are commonly used in Japan. *S. torvum* and *S. sisymbriifolium* protect efficiently eggplant scions from bacterial wilt (Mian et al., 1995). Control of *Verticillium* wilt when using wild *Solanum* spp. is reported by Gao MeiXiu et al. (2001). Mochizuki and Yamakawa (1979a) report that the yield of eggplants grafted on *S. torvum* rootstock is better than when the rootstock is *S. integrifolium* and *S. toxicarium* -i.e. *S. stramonifolium*-. *Solanum* spp. rootstocks can provide other useful advantages such as a lower degree of infestation with *Leucinodes orbonalis,* enhanced photosynthesis and transpiration, resistance to windfall and chilling tolerance (Alam et al., 1994; Gao MeiXiu et al., 2001; Nie LanChun et al. (2004).

Interestingly, eggplant and relatives can also be used as rootstocks for tomato. The Asian Vegetable Research and Development Center (AVRDC) develops *S. melongena* rootstocks resistant to waterlogging as well as to bacterial wilt and other soil born diseases for grafting tomato (Black et al., 2000), and interspecific hybrids [*S. integrifolium* x *S. melongena*] as well as *S. torvum* and *S. sisymbriifolium* are experimented on tomato (Mian et al., 1995).

Since there is a prohibition of methyl bromide for disinfecting soils in Europe, there is a renewed interest for grafting eggplant (and tomato) on rootstocks resistant to soil born pathogens. The tomato rootstocks (mostly interspecific hybrids) used successfully so far are developing new root pathologies and research on new rootstocks for both crops is desirable.

Grafting techniques such as apical or lateral grafts had been carried out on young plants but this was wasteful of time and space. The technique now has been miniaturized by using seedlings. The so called ‘Japanese graft’ uses a decapitated rootstock under or above the cotyledons, to which a scion is affixed and linked by a silicone tube. Within a few days, the two stems are joined. However, this technique reduces strongly the stem length of the rootstock and this can be one of the reasons of the occasional passage to the scion of *Verticillium* then insufficiently retained along the rootstock stem. Zhou BaoLi and Jian Li (2001), Lee (2003), Lee and Oda (2003) review all grafting aspects for eggplant (and other vegetables), including robotization.

5 Genetic Resources

5.1 Stake Holders and Characterization

The International Board for Plant Genetic Resources (IBPGR, renamed International Plant Genetic Resources Institute –IPGRI- in 1991 and Bioversity International in 2006) has included eggplant (in the broad sense) in the list of priority species whose genetic resources suffered genetic erosion, as early as 1977 (Grubben, 1977). In the same momentum, IBPGR published eggplant descriptors in 1988: these descriptors, suitable for all cultivated eggplants, are available online (http://www.ipgri.cgiar.org/). Large collections of several hundreds to over one thousand accessions have been made available by national inputs, in particular in countries where eggplant

is a traditional vegetable, such as in India (collection held by the National Bureau of Plant Genetic Resources, NBPGR, New Delhi,), China (Institute of Vegetables and Flowers, IVF, Beijing), Taiwan (AVRDC) and Japan (National Institute of Agro-biological Sciences, NIAS, Tsukuba). In the 1980s and 1990s IPGRI backed up some national initiatives by sponsoring collecting missions of eggplants in Asian countries such as India, Sri Lanka, Bangladesh, Nepal, Thailand (Wivutvongvana et al., 1984), and Bhutan. The Asian collections contain mostly *S. melongena* accessions, but also some wild or other locally cultivated *Solanum* species. Some of these collections are already on line accessible for passport and characterization data (e.g. http://www.avrdc.org/germplasm.html for AVRDC and http://www.gene.affrc.go.jp/plant/index.html for NIAS).

Collecting missions were also organized under the IPGRI auspices in Middle East, Africa, Mediterranean and other countries (L. Maggioni, pers. com.). The collection in Africa (Lester et al. 1990) dealt with the three cultivated eggplants (*S. melongena*, *S. macrocarpon* and *S. aethiopicum*) as well as with some other locally cultivated species and wild germplasm. Safety duplicates were transferred to the Birmingham Solanaceae collection (University of Birmingham, UK) and to the Center for Genetic Resources (CGN) in The Netherlands (these accessions were regenerated in 2000-2005 by the EGGNET group, see below).

In The United States, USDA assembled an eggplant collection, located in Griffin (Georgia); the passport, description and evaluation data are on line available (http://www.ars-grin.gov). In Mediterranean and continental Europe, many countries have gathered eggplant germplasm: a survey realized in 2000 by the European Cooperative Program for Plant Genetic Resources (ECP/GR) has identified the main stakeholders (in terms of number of accessions) and estimated eggplant genetic resources held in Europe as about 6000 accessions (Daunay et al., 2003). The funding of the Eggplant Network project (EGGNET, 2000-2005) by the European Community has allowed seven countries to join their efforts for coordinating the management of eggplant genetic resources. The main objectives of EGGNET were (1) saving the endangered Solanaceae collection (mostly composed by *Solanum* species, including cultivated and wild eggplants) of the University of Birmingham, (2) regenerating as many accessions as needed and organizing the safety duplication of each collection, (3) defining and using a subset of IPGRI morphological descriptors relevant for breeders and genebanks (primary characterization), (4) defining a set of agronomic traits of interest to breeders, and characterizing accessions for those traits, and (5) establishing a common database containing the passport data (derived from the Multicrop Passport Descriptors) together with the primary and secondary characterization data. The ECP/GR Solanaceae Working Group, initiated by ECP/GR in 2001 concerned by eggplant (and also pepper, tomato, husk tomato and tree tomato) has taken over the legacy of EGGNET. The database became in 2005 the European Central Eggplant database (http://www.bgard.science.ru.nl/WWW-IPGRI/eggplant.htm), and the passport data of the other eggplant collections held in Europe are progressively entered by the curator (Experimental and Botanical Garden of Radboud University, Nijmegen, The Netherlands). For eggplant, as for the other solanaceous crops, the ECP/GR Working Group aims

at coordinating the management of genetic resources at the scale of Europe (taken in the geographical sense).

Given the progressive globalization of genetic resources management, one can expect in the coming years an increasing interaction between eggplant stake holders, which should tend towards inter-connectable databases, better seed exchanges, conservation and safety duplication protocols, as well as a better primary characterization (also said description) of the accessions. Secondary characterization (also said evaluation) of traits of interest, together with the use molecular markers for characterizing genetic diversity will be facilitated by this international cooperation. Thanks to the future on line availability of the databases of most collections, it will become possible to carry out new analyzes such as the redundancy rate and gaps identification between collections, which will help defining regeneration priorities and further collecting efforts.

Eggplant is the only Solanaceae (with potato) to be included in the list of crops covered under the multilateral system defined by the International Treaty (IT) for Plant Genetic Resources for Food and Agriculture, provided they are under the management and control of the Contracting Parties and in the public domain. A standard Material Transfer Agreement (MTA), to be used for facilitated access of genetic resources that are part of the multilateral system, was adopted in June 2006 by the Governing Body of the IT. This means that the international exchange of eggplant genetic resources is facilitated, when compared with the species that are not covered by the multilateral system.

5.2 Intraspecific Diversity

The morphological diversity within and between the wild (Group F), weedy (Group E) and primitive eggplants cultivars (Group G), studied by Lester and Hasan (1991), concerns all plants traits, but in particular plant growth habit (from creeping to erect) and prickliness (from no prickles to many prickles). The fruits have in common small size from (2 to 5 cm in diameter), and a generally round or oblong shape, though finger shaped fruits also exist. Their colour, frequently green, can also match the whole range of colours observed in advanced cultivars, with diverse combinations of anthocyanin and chlorophyll pigments presence and distribution patterns (see section 8.4).

Diversity within *S. melongena* advanced cultivars concerns fruit size, shape and colour but also many plants traits, such as plant vigour (from 20 cm to over 2 m in height), growth habit (from creeping to erect), flowering earliness (from about 55 days after sowing to later than 90 days), life time span (annual to perennial - though this trait is strongly affected by climatic conditions -) anthocyanic pigmentation of vegetative parts (from green to violet), prickliness (from no prickles to many prickles), hairiness (from almost hairless to very hairy), leaf shape (straight to dentate), and so on. Important horticultural traits such as flowering and yield earliness, yield potential (linked to plant vigour), fruit bitterness, climatic adaptation, resistance to pests, diseases and abiotic stresses vary also widely among accessions, but they are far from having been characterized systematically and they deserve much more attention in the future from breeders and germplasm conservators.

IPGRI morphological descriptors and/or "home made" descriptors are used for characterizing the accessions, but they are only sometimes included into the germplasm holders' databases. A minimum set of morphological descriptors to be used by all European stakeholders, in addition to their own descriptors, has been recently set up by the ECP/GR Solanaceae Working Group (http://www.ecpgr.cgiar.org/Workgroups/solanaceae/solanaceae.htm) with the objective of adding these valuable data to the European eggplant passport database.

The analysis of the structuration of *S. melongena* diversity for morphological traits completed by molecular markers (Amplified Fragment Length Polymorphisms; AFLPs) has been carried out on a collection of 28 Spanish landraces at the Polytechnic University of Valencia, Spain (Prohens et al., 2005). This approach deserves to be extended in the future to wider collections for a better understanding of the genetic diversity available to breeders. Although based on modest sets of accessions (<100), estimations of the intraspecific diversity have been carried out for isozymes (Isshiki et al., 1994b), allozymes (Karihaloo and Gottlieb, 1995), RAPIDs (Karihaloo et al. 1995), and AFLPs (Furini and Wunder, 2004). Various markers of the intra-specific diversity are under active investigation, for instance for tri-nucleotide microsatellites (Nunome et al., 2003a), Sequence Tagged Microsatellite Site (STMS) (Behera et al., 2006) and Random Amplified Polymorphic DNA (RAPDs) (Singh et al., 2006).

5.3 *Solanum* Species Related to *S. melongena* and Interspecific Crossability

Though considerable progresses have been made for 30 years in the understanding of eggplant relatives, the knowledge of the germplasm related to eggplant is still incomplete, and this for many reasons. First, the genus *Solanum* is the largest genus of the Solanaceae family and one of the largest plant genera. It contains approximately 1400 species (Bohs, 2005), which were described since Linnaeus by a plethora of species names (about 5000!), with frequent homonymies and synonymies. This renders the stabilization of species identification and nomenclature even more complicated. The genus *Solanum* has been subdivided by D'Arcy (1991) into 7 subgenera including subgenus *Leptostemonum* to which *Solanum melongena* and its related species belong to. Over 400 species belong to this subgenus which was tentatively structured in 34 groups *sensu* Whalen (1984), and later on in 27 sections *sensu* D'Arcy (1991) and 10 clades (Levin et al., 2006).

Taxonomic approaches based on restriction site and sequence data across the entire family (Olmstead and Palmer, 1992; Olmstead et al., 1999), within the genus *Solanum* (Olmstead and Palmer, 1997, Bohs and Olmstead, 1997, 1999; Bohs, 2005; Bohs, 2006) and more recently within the subgenus *Leptostemonum* (Levin et al., 2006) bring new information about the phylogenetic relationships across taxa. In addition to these purely taxonomic researches, molecular diversity studies on eggplant and related species, more aimed at evaluating genetic distances than at real taxonomic purposes, are also carried out by geneticists, such as those using seed storage proteins (Mennella et al., 1999; Karihaloo et al., 2002), allozymes (Isshiki et al., 1994a), chloroplastic DNA (Sakata et al., 1991; Sakata and Lester, 1997);

Restriction Fragment Length Polymorphism (RFLPs) from chloroplastic DNA (Isshiki et al., 1998) and mitochondrial DNA (Isshiki et al., 2003), trinucleotide microsatellites (Nunome et al., 2003a), AFLPs (Furini and Wunder, 2004), STMS markers (Behera et al., 2006), and RAPDs (Singh et al., 2006).

Despite the moving taxonomic context, a first tentative list of the *Solanum* species related to eggplant was set up (Daunay et al., 1999) and yielded some 120 putative distinct taxa. The taxonomic treatment of the African *Solanum* species unfortunately was not completed by Lester and Jaeger (Jaeger, 1985); however, the Planetary Biodiversity Inventory (PBI) *Solanum* project has taken over this task and will undoubtedly identify some more African species so far mixed up in the mass of confusing nomenclature, thanks to herbarium specimen comparisons and to phylogenetic researches.

The second difficulty is due to the fact that only about half of the species putatively closely related to eggplant (about 120) are available in germplasm collections (about 60), and this of course hinders the taxonomic studies as well as the interspecific crosses experiments. Indeed, from the breeding point of view, a promising close relationship between *S. melongena* and any *Solanum* sp. is based on their crossability and on the obtention of fertile progenies from the interspecific hybrid. The interspecific sexual crosses carried out so far are summarized in Table 2. However, this Table provides only rough information. Indeed, the results obtained by different authors can not be compared ideally to each other because of the different ways (i) a given cross was attempted (several accession of the partner species used *versus* a single parental combination; cross made in one direction only *vs.* cross made in both directions with *S. melongena* used as female as well as male; use of in vitro embryo rescue vs. no use) and (ii) the hybrid fertility was assessed (use of pollen stainability for estimating the hybrid fertility vs. no use; attempt of obtaining progenies from the interspecific hybrid vs. no attempt). Therefore the results displayed in Table 2 should not be considered as definitive. The so far unsuccessful crosses displayed in this Table might succeed in the future, in particular if the following precautions are taken: (1) several accessions of each parental species used, (2) reciprocal crossing, and (3) use of in vitro embryo rescue.

Given all these reserves, Table 2 is still indicative of some important information. On the whole 63 *Solanum* species (belonging to 14 sections sensu D'Arcy) have been tentatively sexually hybridized with *S. melongena*. The results obtained vary from (1) no fruit set, to (2) parthenocarpic fruit set, (3) early death of the embryos, (4) abnormal hybrid plants, (5) normal hybrid plants setting abnormal seeds or seeds which do not germinate, (6) virtually sterile hybrids with pollen stainability <10%, (7) partially fertile hybrids with 10% to 50 % pollen stainability, and (8) fertile hybrids with 50% to 100 % pollen stainability.

If using the minimum threshold of 10% pollen stainability for declaring a given cross successful, then the results obtained so far indicate that 27 species are successfully crossable with eggplant. They belong only to the sections *Melongena* (11 species out of 27) and *Oliganthes* (15 species out of 27), with one exception for *S. lidii* which belongs to section *Nycterium*. One should notice however, that several species of both these sections *Melongena* (e.g. *S. cinereum*) and *Oliganthes* (e.g. S. *lamprocarpum*) do not hybridize successfully with *S. melongena*; hence the fact

that a given species belongs to one of these sections is not at all a guarantee of its crossability with *S. melongena*.

Table 2. Results of the interspecific crosses between *S. melongena* and related species (from Daunay *et al*., 1991; Daunay *et al*., 1998; Daunay *et al.*, 1999; Lester and Hasan, 1991; Daunay, unpub. results). The cross direction is not specified.
Hybrid virtually sterile: pollen stainability < 10%
Hybrid partially fertile: 10% < pollen stainability < 50 %
Hybrid fertile: 50% < pollen stainability < 100%.

Section	Species	Best result when hybridized with *S. melongena* L.[a]	Seed set of interspecific hybrid[b]
Acanthophora Dunal	*S. capsicoides* All.	No germination of seeds	
	S. mammosum L.	Abnormal seeds	
	S. *viarum* Dunal [c]	Hybrid virtually sterile	
Anisantherum Bitter	S. *pubescens* Willd.	Hybrid virtually sterile	
Campanulata Symon	*S. campanulatum* Symon	Abnormal seeds	
Croatianum D'Arcy & Keating	*S. heinianum* D'Arcy & Keating	No fruit set	
Cryptocarpum Dunal	*S. sisymbriifolium* Lam.	Death of hybrid seedlings	
Ischyracanthum Bitter	S. *dennekense* Dammer	No fruit set	
Lasiocarpa (Dun.) D'Arcy	*S. stramonifolium* Jacq.	No fruit set	
Melongena (Mill.) Dun.	*S. aculeastrum* Dunal	Parthenocarpic fruits	
	S. beaugleholei Symon	Parthenocarpic fruits	
	S. cerasiferum Dunal	Hybrid partially fertile	Normally shaped seeds
	S. chippendalei Symon	Death of hybrid seedlings	
	S. *cinereum* R. Br.	Hybrid virtually sterile	
	S. *clarkiae* Symon	No viable hybrid embryos	
	S. dasyphyllum Thonn.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
	S. dioicum W.V. Fitz.	Parthenocarpic fruits	

	S. *diversiflorum* F. Muell.	Parthenocarpic fruits	
	S. incanum group A (S. *campylacanthum* Hochst. ex A. Rich.)	No germination of seeds	
	S. incanum group B (*S. delagoense* Dunal) [d]	Hybrid fertile	Viable progenies obtained
	S. incanum L. sensu stricto (group C)	Hybrid fertile	
	S. incanum group D (*S. lichtensteinii* Willd.)	Hybrid fertile	
	S. linnaeanum Hepper & Jaeger	Hybrid partially fertile	Viable progenies obtained
	S. macrocarpon L.	Hybrid partially fertile	Viable progenies obtained
	S. *marginatum* L. fil.	Hybrid virtually sterile	
	S. *melanospermum* F. Muell.	Hybrid partially fertile	No seeds
	S. phlomoides A. Cunn. ex Benth.	No viable hybrid embryos	
	S. *richardii* Dunal	Hybrid partially fertile	Mixture of normally shaped & empty seeds
	S. sessilistellatum Bitter	Hybrid partially fertile	Mixture of normally shaped & empty seeds
	S. tudununggae Symon	Parthenocarpic fruits	
	S. virginianum L.[e]	Hybrid partially fertile	
Monodolichopus Bitter	S. *coagulans* Forssk. (*S. dubium* Fresen.)	Hybrid virtually sterile	Empty seeds
Nycterium (Ventenat) Dunal	*S. lidii* Sunding	Hybrid partially fertile	Mixture of normally shaped & empty seeds
	S. verspertilio Ait.	No fruit set	
Oliganthes (Dunal) Bitter	S. aethiopicum L.[f]	Hybrid partially fertile	Viable progenies obtained
	S. anguivi Lam. (*S. indicum* auct. non L. p.pte)	Hybrid partially fertile	
	S. burchellii Dunal	Hybrid partially fertile	Mixture of normally shaped & empty seeds
	S. capense L.	Hybrid virtually	Mixture of normally

	sterile	shaped & empty seeds
S. catombelense Peyr.	Hybrid partially fertile	Mixture of normally shaped & empty seeds
S. *coccineum* Jacq.	Hybrid partially fertile	Mixture of normally shaped & empty seeds
S. cyaneopurpureum De Wild.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. *dinteri* Bitter	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. forskalii Dunal (=*S. albicaule* Kotschy ex Dunal)	Hybrid virtually sterile	No seeds
S. *hastifolium* Hochst.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. kurzii Brace & Prain (*S. sanitwongsei* Craib.)	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. lamprocarpum Bitter[g]	Hybrid virtually sterile	No seeds
S. myoxotrichum Baker	Parthenocarpic fruits	
S. pyracanthos Lam.	No germination of seeds	
S. *rigescens* Jacq.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. rigescentoides Hutch.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. rubetorum Dunal (*S. rigescens* auct. non Jacq.)	Hybrid partially fertile	
S. *supinum* Dunal	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds
S. toliaraea D'Arcy & Rakotozafy	Parthenocarpic fruits	
S. tomentosum L.	Hybrid partially fertile	Mixture of normally shaped seeds and empty seeds

	S. trilobatum L.	Parthenocarpic fruits	
	S. violaceum Ort. (*S. indicum* auct. non L. p. pte)	Hybrid partially fertile	
Stellatipilum sensu Seithe	S. *grandiflorum* Ruiz & Pavon (*S. macranthum* Dunal)	Hybrid virtually sterile	
Torva Nees	*S. giganteum* Jacq.	Parthenocarpic fruits	
	S. goetzii Dammer	Hybrid virtually sterile	No seeds
	S. hispidum Pers. (*S. warscewiczii* Weick. ex Lambertye)	Hybrid virtually sterile	
	S. schimperianum Hochst.	Parthenocarpic fruits	
	S. torvum Sw.	Hybrid virtually sterile	Viable progenies obtained [h]
Unknown	*S. mahoriensis* D'Arcy & Rakotozafy	No viable hybrid embryos	

[a]when an interspecific cross is realized, various results may be obtained: no fruit set on the mother plant used for the cross, obtention of parthenocarpic fruit, fruit set but the seeds do not germinate. When early embryo rescue is performed, the hybrids may behave in different ways: the seedlings die, or they survive and produce abnormal plants or normal plants. In this latter case hybrid plant displays various fertility levels, assessed here by pollen stainability.

[b]the results are available either in terms of seed set of the interspecific hybrids (those seeds having not been sown so far), or in terms of viability of the progenies (OP or controlled F_2 or BCs) when the seeds were sown.

[c]synonyms: *S. khasianum* Clarke var. *chatterjeeanum* Sengupta, *S. xanthocarpum* auct. non Schrad. & Wendl.

[d]this species was misnamed *S. panduriforme* in Daunay *et al*. 1991, 1999, 2001 and in Lester and Hasan, 1991.

[e]synonyms: *S. xanthocarpum* Schrad. & Wendl.; *S. surratense* Burm. F.

[f]we provide here results without giving the details per *S. aethiopicum* cultigroup. *S. aethiopicum* as given here includes Aculeatum Group (often named *S. integrifolium*), Gilo, Kumba and Shum Groups.

[g]this species was misnamed *S. zanzibarense* Vatke in Daunay *et al*., 1998 and 1999.

[h]various progenies were obtained at INRA after tetraploidization of the interspecific hybrid with colchicine treatment, but so far they are sterile (Daunay, 1989-90; Daunay et al., 1999).

The case of *S. capense* and *S. melanospermum* is instructive. The interspecific hybrid between *S. capense* and eggplant is virtually sterile with less than 10% of stainable pollen, but is however able to set fruits with seeds; though part of these

seeds is empty and part is normal, one can expect a possibility to obtain progenies from this hybrid. Conversely, *S. melanospermum* yields a partially fertile hybrid (10 to 50% pollen stainability) when crossed with eggplant, but this hybrid does not set seeds; hence it does not seem possible to obtain progenies from it. These two examples, together with the various reserves expressed above, illustrate how convoluted is the path before one can rule out the impossibility of a given interspecific cross between eggplant and a given *Solanum* species.

The results provided in Table 2 indicate also that the rate of success of interspecific crosses involving eggplant and putatively related species, if estimated on the basis of a hybrid pollen stainability > 10%, is about 43% (27 successful out of 63 carried out). This means that one can theoretically expect about 40-50 *Solanum* species (out of the over 100 species listed indicatively by Daunay et al. (1999) which are potentially able to be successfully hybridized with *S. melongena*. This wide reserve of genetic variability is of course very promising for the future genetic improvement of eggplant, and will keep future scientists busy at attempting interspecific crosses, and characterizing the wild germplasm for desired horticultural traits.

Interspecific somatic hybridization has also been used successfully, but only with a small number species (*S. sisymbriifolium*, *S. torvum*, *S. khasianum* - i.e. *S. viarum*-, and *S. aethiopicum*), and so far, it did not succeed at obtaining progenies from the somatic hybrids in the cases for which their sexual counterparts failed (see section 10.2).

5.4 Gene Pools

Primary genepool sensu Harlan and De Wet (1971) consist of *S. melongena* germplasm, including wild forms (groups E, F, G) as well as advanced cultivars (group H), which are all interfertile. The secondary genepool consist of species which are crossable with *S. melongena*, and the tertiary gene pool comprises species very difficult or impossible to cross with it. However for the many reasons developed (see section 5.3) the allocation of *Solanum* spp. to these secondary and tertiary genepools is quite risky because of an incomplete knowledge of the species concerned and of their behaviour when crossed with eggplant. Further, the limits between the primary, secondary and tertiary genepools are blurred since the behaviour of species when hybridized with eggplant, does not match exactly the limits as defined by Harlan and De Wet. Indeed, for example, some wild species such as *S. incanum* groups C and D, as well as *S. cerasiferum* or *S. sessilistellatum* cross relatively easily with eggplant (though we have not observed so far spontaneous crosses) and the interspecific hybrids yield progenies which exhibit relatively few sterility troubles (Table 2). Hence such species could almost be included in the primary genepool. Other examples are the case of *S. torvum* which so far yields sterile hybrids whatever they are obtained by sexual or somatic hybridization with eggplant, and of *S. sisymbriifolium* which yields a hybrid only by using somatic hybridization: those species can be considered as standing at the limit between the secondary and the tertiary genepool.

6 Disease Resistance

In practice, the commercial cultivars display a poor spectrum of disease resistances, when compared with other solanaceous crops such as tomato and pepper. The intra-specific and inter-specific diversity for disease resistance is far from being systematically evaluated, but sources of resistance against various diseases have already been described, and used for breeding. A review of eggplant diseases and pests has been done by Daunay (2007) and the review of resistances described in the literature by Daunay et al. (1991, 2001b) and Robinson et al. (2001). The confusing nomenclature of *Solanum* species in the literature relative to diseases and pest resistance renders difficult the understanding of the real species tested. In the updated synthesis of the resistance of eggplant and its relatives to diseases and pests that is presented hereafter, we have indicated, when possible, the putative correct species name next to the one used by the authors.

The systematic evaluation of the intra and inter-specific germplasm to a set of proprietary pests and diseases is desirable in the future because it would yield a useful knowledge for breeders as well as a better understanding of resistances frequency and geographical origin(s) within the germplasm held at least in the main collections. Such last scale project can be realized through international collaborations between germplasm holders and breeders.

6.1 Bacterial Wilt, *Ralstonia solanacearum* Smith (*Pseudomonas solanacearum*)

Bacterial wilt is a widespread soil borne tropical disease which invades the plant vessels and provokes the complete wilting of the plant followed by its death. Resistance to this disease is by far the most frequent resistance carried by eggplant tropical cultivars, but the strong interaction between the bacteria strains, the agro-climatic conditions and the efficiency of the resistances available poses a problem, amplified by the internationalisation of the vegetables seed trade. Various methods of inoculation are used, including natural field infestation, root cut inoculation, and stem pricking (at the leaf axil with an inoculated sharp tool) and the common record system for resistance is the wilting percentage of the accessions tested. However, it is preferable to use a more precise phenotyping method giving access to the bacteria invasiveness and density at the collar and upper stem levels since symptomless plants are latently infected at the collar level (Grimault et al., 1994). The resistance mechanism in eggplant appears to be similar to tomato, which is the limitation of the spread of the pathogen within the stem: the more resistant a plant, the lower the stem colonization (Grimault and Prior, 1994). Gopalakrishnan et al. (2005) observed in resistant cultivars particular features of the root cortical cells (small and tightly packed), as well as high phenolic content in the roots which both could prevent the entry and further multiplication and spread of the bacteria.

Various degrees of varietal resistance have been reported by many authors (e.g. Messiaen, 1975; Mochizuki and Yamakawa, 1979b; Chen NungChe et al., 1997). Depending on the varieties used, resistance to *Ralstonia solanacearum* is found monogenetically and dominantly (e.g. Chadha, 1993; Chaudhary, 2000) or recessively

inherited (Chaudhary, 2000; Gopalakrishnan et al. 2005), polygenic dominant (e.g. Ano et al., 1991; Chaudhary, 2000) or polygenic recessive (e.g. Feng LinLin et al., 2003). Gousset et al. (2004) found that cytoplasmic factors could interfere. From this rapid survey, one can gather that several mechanisms of resistance are probably available within eggplant germplasm. In the case of a monogenic dominant resistance one RAPD marker located at 4,33 CM from the gene, was obtained by Zhu HuaWu et al., 2005.

The recent structuration of the genetic diversity of *Ralstonia solanacearum* into several distinct phylotypes (Prior and Fegan, 2005; Fegan and Prior, 2005) will help breeders to better control the interactions between the resistances they breed for and the bacterial strains they use in their breeding programs, and hence to better understand the genetic control of the resistance(s).

Eggplant related germplasm has been only partially but already successfully investigated and sometimes also used as rootstocks. The highest level of resistance is found in *S. torvum* and *S. toxicarium* -i.e. *S. stramonifolium*- (Hébert, 1985; Mochizuki and Yamakawa, 1979 a and b; Mondal et al., 1991). Gousset et al. (2005) report that the resistance of *S. torvum* (few or no wilting of plants) is found in several accessions, but that the bacteria is present (serological test) in the roots of symptomless plants; this suggests that the mechanism of resistance of this species is tolerance. Date et al. (1994) point out that the resistance of *S. torvum* can be broken down by a combination of some bacterial strains (belonging to group IV) and high ambient temperatures.

The resistance of *S. integrifolium* (i.e. *S. aethiopicum* Aculeatum Group) to this disease -and to *Fusarium* wilt, see section 6.2- is known for a long time in Japan; it was also found efficient in the French West Indies conditions (Hébert, 1985). However, there are susceptible accessions in *S. aethiopicum* Aculeatum Group (Monma et al., 1996). Within *S. aethiopicum* Gilo Group (same species but another cultigroup) and *S. anguivi* (its closest wild relative) some resistant accessions were identified by Hébert (1985), but not by Monma et al. (1996) who found susceptible all the accessions they tested. Collonnier et al. (2001a) transferred the resistance of two accessions of *S. aethiopicum* into *S. melongena* by somatic hybridization (see section 10.2).

Other various levels of resistance were found in a wild type of eggplant *–LS 174-* (Hébert, 1985), *S. sisymbriifolium* (Mochizuki and Yamakawa, 1979b; Mondal et al., 1991), *S. viarum* (Hébert, 1985), *S. xanthocarpum* from India - possibly *S. viarum* - (Mochizuki and Yamakawa, 1979b), *S. warzcewiczii* - *S. hispidum* - (Hébert, 1985), *S. mammosum* (Mondal et al., 1991) and *S. surratense* - *S. virginianum* - (Mondal et al., 1991). Resistances were also found in *S. nigrum* and *S. maroniense* (Mochizuki and Yamakawa, 1979b), but these species are so distantly related to eggplant that they cannot be used in breeding programs; they keep however a potential interest as rootstocks, contingent on their graft affinity with eggplant. Sakata et al. (1989) identified resistance in *Solanum violaceum*, as well as in distant species (*S. carolinense*, *S. pseudolulo*, *S. tequilense*).

In the future, the efficiency for investigating the germplasm of eggplant and relatives for resistance to bacterial wilt needs to be improved by the better knowledge of the strains/phylotypes used for the resistance tests, by the use of an

appropriate method for phenotyping resistance, and also by a better use of the *Solanum* nomenclature.

6.2 Fusarium Wilt, *Fusarium oxysporum* f.sp. *melongenae*

Fusarium wilt on eggplant is a serious soil born disease in Japan which induces vascular disorders, vessels browning and subsequent wilting of the plants. Its occurrence in Europe is rather recent; it was described in the Netherlands (van Steekelenburg, 1976), Italy (Stravato et al., 1993), Spain (Urrutia Herrada et al., 2004) and Turkey (Altinok, 2005). Messiaen (1975) and Yamakawa and Mochizuki (1979) reported varietal differences for various resistance levels, but complete resistance was not observed. Abdullaeva and Shifman (1988) identified high levels of resistance in several varieties that they used successfully in breeding programs. Mochizuki et al. (1997) described a monogenic dominant resistance in the line *LS174*. Yoshida et al. (2004 a, b) and Monma et al. (1997) developed eggplant rootstocks resistant to *Fusarium* wilt, and also resistant to bacterial wilt.

Within the germplasm related to eggplant, Yamakawa and Mochizuki (1979) found complete resistance in *S. integrifolium* (i.e. *S. aethiopicum* Aculeatum Group), and *S. indicum* (i.e. probably *S. violaceum*), as well as in *S. incanum* (probably a wild form of *S. melongena*) which however segregated into resistant and susceptible plants. Cappelli et al. (1995) found (1) that *S. torvum* and *S. sisymbriifolium* were resistant, (2) that *S. integrifolium*, *S. aethiopicum* Gilo Group, *S. khasianum* -i.e. *S. viarum*- and *S. macrocarpon* segregated for resistance, and (3) that *S. sodomeum* (i.e. *S. linnaeanum*) was susceptible. Monma et al. (1996) found that 96% of the 50 accessions of *S. gilo* and *S. aethiopicum* (same species), and 100% of the *S. anguivi accessions* (7) they tested expressed total resistance, thus clearly indicating that *Fusarium* wilt resistance is very frequent in this African species and its close wild relative. They found also resistance in *S. macrocarpon*.

6.3 Verticillium Wilt, *Verticillium dahliae* Kleb. and *V. albo-atrum* Beinke & Berth.

Verticillium is another vascular disease, which likewise the *Fusarium* wilt provokes vessels browning and foliar wilting, but it kills rarely the plants; however it reduces significantly the yield. Vigouroux and Molot (1975) established a relationship between the susceptibility of eggplant varieties and their sugar content. Methods for assessing the resistance include naturally infected fields or re-enforced inoculation in open field, and plantlets' root dipping into a solution of inoculum. Symptoms measurement most often focus on foliar wilting, sometimes on vessels browning spread along the stem -which is the most relevant criteria-, and more rarely on plant height reduction.

Though actively searched for, resistances of high level to *Verticillium* wilt within eggplant germplasm are scarcely reported (Petrov et al., 1989; Bletsos et al., 2004). Lesser susceptibility or tolerance (Nothman and Ben Yephet, 1979; BaiQing Lin YunHua Xiao, 1995; Neshev et al., 1997; Robinson et al., 2001) or the so called

'slow wilting' (Cirulli et al., 1990) are reported in several varieties. The existence of particularly susceptible accessions is reported by Cirulli et al. (1990).

The germplasm of *Solanum* species was of course actively screened for resistance to *Verticillium* wilt, but this search and its survey are hampered by many factors, including the difficulty of being sure of the identity of the botanical material since the use of inappropriate names is very common in the literature. Alconero et al. (1988) reported relatively high resistance levels in *S. torvum*, *S. sisymbriifolium*, *S. aculeatissimum* and *S. scabrum*. The resistance of *S. torvum* and *S. scabrum* was confirmed by Sakata et al. (1989), who found also resistances in far related species (*S. caripense* and *S. persicum*). BaiQing Lin and YunHua Xiao (1995) found that one accession of *S. aethiopicum* and one of *S. coagulans* had similar resistance levels than *S. sisymbriifolium*. Robinson et al. (2001) on the basis of foliar symptoms found resistant accessions in many species (*S. torvum*, *S. sisymbriifolium*, *S. aculeatissimum*, *S. scabrum* already described as resistant -see above- , as well as *S. aculeastrum*, *S. americanum*, *S. anguivi*, *S. atro-purpureum*, *S. capsicoides*, *S. ciliatum*, *S. crinitipes*, *S. glutinosum*, *S. hartwegii*, *S. hispidum*, *S. incanum*, *S. khasianum*, *S. linnaeanum*, *S. lividum*, *S. marginatum*, *S. nigrum*, *S. panduriforme*, *S. tomentosum*, *S. viarum* and *S. villosum*). Yoshida et al. (2004c) report the resistance of an interspecific hybrid between *S. grandifolium* (i.e., it probably refers to *S. grandiflorum*, given that *S. grandifolium* is a South American species and does not hybridize easily with eggplant) and *S. melongena*, that is resistant to *Verticillium* wilt.

The resistance of *S. sisymbriifolium* and *S. torvum* could not be so far transferred to eggplant because their sexual cross with eggplant either did not yield progenies or yielded sterile progenies (see Table 2). Somatic crosses were realized for both species, with so far limited success in terms of breeding for resistance since the somatic hybrids are sterile (see section 10.2).

Resistance of *S. sodomeum* (= *S. linnaeanum*) was reported by Pochard and Daunay (1977/78), though later experiments (Daunay et al. unpubl.) showed that this species presents few wilting but strong vessels browning, thus depending on the criterion used for assessing its resistance, it can be considered as more or less resistant. However that may be, Acciarri et al. (2001) reported the introgression of the resistance of this species into *S. melongena* through interspecific hybridization and advanced back cross material was screened for resistance in the field conditions (Acciarri et al., 2004). Sunseri et al. (2003) developed AFLPs for tagging the resistance factors of these progenies.

Alconero et al. (1988) reported susceptibility in *S. gilo* (i.e. *S. aethiopicum* Gilo Group), *S. integrifolium* (*S. aethiopicum* Aculeatum Group), *S. incanum, S. macrocarpon*, *S. laciniatum*, *S. mammosum* and *S. nodiflorum*. Monma et al. (1996) found that a large set of *S. aethiopicum* accessions, as well as two accessions of *S. macrocarpon* were susceptible. Robinson et al. (2001) found susceptible all the accessions they tested for *S. aethiopicum* (named *S. aethiopicum*, *S. gilo*, *S. integrifolium*, *S. olivare*), *S. cinereum*, *S. indicum* (possibly *S. violaceum*), *S. laciniatum*, *S. macrocarpon*, *S. mammosum*, *S. nodiflorum*, and *S. surratense* (*S. virginianum*).

Resistance to *Verticillium* wilt, one of the major pathological problems of eggplant, is still a challenge for eggplant breeders, because they do not have high levels of resistance available in eggplant germplasm, and they could not transfer so far those present in wild species such as *S. torvum* and *S. sisymbriifolium* because of interspecific crossing incompatibilities. In tomato, this disease is controlled by the monogenic dominant genes Ve1 and Ve2. Interestingly, Fei JiOng et al. (2004) have cloned and characterized a Ve like gene (*StVe*) from *S. torvum*, thus demonstrating some parallelism between the two species for the control of the disease. Given the high synteny between the genomes of tomato and eggplant, the reasons of the absence of a functional gene controlling *Verticillium* wilt in eggplant could possibly be cleared out in the future by a detailed molecular investigation of the part of its genome where such a Ve like gene should be located. So long wild germplasm or genetic engineering do not bring to breeders workable high level resistances, the use in breeding of eggplant genitors carrying lesser susceptibilities to *Verticillium* wilt remains a temporary and valuable solution. Indeed, recurrent selection schemes (see section 9.3) can probably yield transgressive resistance levels via the recombination of favourable minor genes.

6.4 Other Fungi

According to Messiaen (1975) resistance to fruit anthracnosis, caused by *Colletotrichum gloeosporioides* f.sp. *melongenae,* is quite common within *S. melongena* germplasm; it is monogenetically controlled (Kaan, 1973). *S. torvum* is a natural host of this fungus (Messiaen, 1975). Chauhan and Duhan (1980) report resistance against *Alternaria melongenae*, *A. solani*, *Phomopsis* sp., *Cercospora solani-melongenae*, and *C. solani* within eggplant germplasm. Immunity through hypersensitivity to *Cercospora solani* has been found in eggplant as well as in *S. macrocarpon* by Madalageri et al. (1988). The resistance to *Phomopsis* blight and fruit rot within *S. melongena* germplasm was shown as polygenic and recessive (Kalda et al., 1977). Wild species were found resistant to this fungus such as *S. indicum* (probably *S. violaceum*) and *S. khasianum* -probably *S. viarum*- (Vadivel and Bapu, 1989), *S. torvum*, *S. khasianum* as well as *S. xanthocarpus* -unknown species- (Datar and Ashtaputre, 1988).

High level of resistance against powdery mildew (*Oidiopsis* [*Leveillula*] *taurica*, consisting of 0-5% of infected leaf surface has been reported (Mahrshi et al., 1980). High as well as moderate resistance levels of eggplant lines against *Sclerotinia sclerotiorum* have been revealed by artificial inoculation (Kapoor et al., 1989/1990) and only moderate resistance against *S. rolfsii* were found (Begum and Ahmed, 1990).

6.5 Viruses

Several viruses transmitted by insects (e.g., CMV, AMV, PVY, TSWV, EDMV, Begomoviruses) or by contact (e.g., TMV, ToMV), can infect eggplant, but in practice their occurrence is much more limited than on other Solanaceae such as pepper and tomato. Reports of search for virus resistance are scarce in the literature.

Potato virus Y (PVY) is transmitted by different aphid species. As shown by Marchoux et al. (unpub. results) very few eggplant genotypes are susceptible to PVY but related species are susceptible such as *S. aethiopicum*, *S. macrocarpon* and *S. sisymbriifolium* (Daunay et al., 2004a). The heredity of the resistance was studied by Daunay et al. (2004a) on F1, F2 and BCs progenies obtained from a cross between *S. linnaeanum* (susceptible) and *S. melongena* (resistant). A single recessive gene *ptv-1* responsible of the resistance in eggplant (line MM 738) was identified, but work is still in progress for ascertaining this result (Caranta et al., unpub. results).

Tomato spotted wilt virus (TSWV) is transmitted by the Thrips *Frankliniella occidentalis*. Pédron et al. (2001) identified hypersensitive response (local lesions) in eggplant lines *MM 27* and *MM 136* when inoculated at the 2-4 leaves stage and this result was confirmed by Dussault-Pelain et al. (unpub. results). Both authors demonstrated a strong effect of plant age on the expression of this resistance. For instance *MM 27* and *MM 136* are resistant at the 2-4 leaves stages, but susceptible at the cotyledon stage. Varietal differences for resistance to TSWV were found in Brazil (Lima et al., 2002). Pédron et al. (2001) found that among a collection of 20 wild *Solanum* species only *S.hastifolium* and *S. sisymbriifolium* were resistant (symptomless and ELISA negative).

Tobamoviruses are transmitted by contact. Rast (1991), screening 526 eggplant accessions for resistance to tobamoviruses, identified hypersensitive reactions (local necrotic reactions) towards TMV and ToMV. Daunay et al. (2004a) showed that the resistance of the line *MM136* to ToMV pathotype 0 was controlled by a dominant gene named *Tbm-1* and that heterozygous genotypes suffered generalized systemic necrosis, a reaction also present in tomatoes heterozygous for the Tm2 gene. Tzortzakakis et al. (2006) found resistance to three tobamoviruses (TMV, ToMV and PMMV) in accessions of *S. aethiopicum* Gilo Group, while *S. aethiopicum* Kumba group, *S. linnaeanum*, *S. dasyphyllum* and *S. macrocarpon* were susceptible.

6.6 Phytoplasma

Little leaf disease, often described in Indian eggplant literature, is transmitted by jassids such as *Amrasca devastans* (Dist.) (also named *Empoasca devastans*) and *Hishimonus phycitis* (Dist.) (also named *Eutettix phycitis*). Using graft inoculation, Chakrabarti and Choudhury (1975) found resistance in the eggplant line S212-1, which is rich in ascorbic acid. Field resistance was evidenced within *S. melongena* germplasm by Chauhan and Duhan (1980). Through graft inoculation, *S. integrifolium* (i.e. *S. aethiopicum* Aculeatum group) and *S. gilo* (probably *S. aethiopicum* Gilo Group), rich in phenolic content, reacted by a hypersensitive reaction and the interspecific hybrids realized with eggplant behave like their resistant parent (Chakrabarti and Choudhury, 1975). Under natural field conditions, Warade et al. (2004) observed no incidence of little leaf disease (and lower infestation rates of the vectors, see section 7.3) on *S. indicum* (i.e. probably *S. violaceum*), *S. insanum* (weedy type of eggplant), *S. integrifolium* and *S. gilo* and on several interspecific hybrids issued from these species and eggplant cultivars.

Eggplant yellowing is another phytoplasmic disease that causes a sudden growth stop, a foliar epinasty and the yellowing of the whole plant that dies within 3 weeks. It is quite common in the South of France. No resistance has been reported so far.

6.7 Root Knot Nematodes, *Meloidogyne* spp.

Several *Meloidogyne* species can infest eggplant. The entire collection of the US Department of Agriculture was screened for resistance to *M. incognita* by Fassuliotis (1973) but no resistances were found. Nandwana et al. (1980) did not find either resistance to *M. incognita* within *S. melongena* germplasm they screened, but they indicated that the number of nematodes eggs was a more reliable criterion for determining plant response than the number of galls per gram of root. On the basis of the number of nematodes juveniles and adults per root system 3 months after inoculation, Reddy et al. (1986) identified resistance in the lines *Maroo marvel* and *BR 112*.

Upadhyay et al. (1977) screened numerous cultivars for resistance to *M. javanica* and found that no variety was immune, but that there were strong varietal differences for susceptibility (from 25% galling to 100%). Boiteux and Charchar (1996) identified complete resistance (no nematode egg-mass formation 7 weeks after inoculation) in the line *A-264-A* originating from Philippines, and partial resistance in several commercial genotypes.

Concerning the related *Solanum* species, Messiaen (1975) reported the high resistance level of *S. torvum* to *Meloidogyne* spp. that Hébert (1985) and Daunay and Dalmasso (1985) confirmed. The resistance of *S. sisymbriifolium* to *M. incognita* reported by Fassuliotis (1973) was confirmed by Daunay and Dalmasso (1985), who however pointed out a lesser resistance level of this species than *S. torvum*, as well as a heterogeneous behaviour among the plants. Daunay and Dalmasso (1985) identified also a high level of resistance in *S. warzcewiczii* (*S. hispidum*). *S. torvum*, *S. sisymbriifolium* and *S. warzcewiczii* were shown to be less resistant towards *M. arenaria* and even less towards *M. javanica. However,* for *S. torvum* Boiteux and Charchar (1996) reported resistance to *M. javanica*.

Daunay and Dalmasso (1985) as well as Tzortzakakis et al. (2006) found that all accessions of S. aethiopicum tested were susceptible, whatever the *Meloidogyne* species used. Ali et al. (1992) noted also the susceptibility of *S. integrifolium* (i.e. probably *S. aethiopicum* Aculeatum group). However, Hébert (1985) identified relatively good levels of resistance to *M. incognita* in some accessions of *S. aethiopicum.* Such discrepancies between results obtained on a given *Solanum* species may have various causes, in particular the different accessions tested, and different methods used for assessing the degree of resistance (e.g. field trial *vs.* artificial inoculation of young plants, number of galls *vs.* number of egg masses).

Immunity or high resistance to *M. incognita* of *S. khasianum* (i.e. probably *S. viarum*), *S. toxicarium* -i.e. *S. stramonifolium*-, and again *S. torvum* is reported by Ali et al. (1992). These authors noted also the susceptibility of *S. indicum* (i.e. *S. violaceum*), as well as the great susceptibility of *S. surratense* (i.e. probably *S. virginianum*) and *S. mammosum*. Several other *Solanum* species (*S. macrocarpon*, *S. aculeatissimum*, *S. xanthocarpon* (i.e. *S. virginianum*), *S. sodomeum* (i.e.

S. linnaeanum) were shown to be susceptible to various degrees to *M. incognita*, *M. arenaria* and *M. javanica* by Daunay and Dalmasso (1985). Tzortzakakis et al. (2006) confirmed the susceptibility of *S. macrocarpon*, *S. linnaeanum* and found *S. dasyphyllum* -wild progenitor of *S. macrocarpon*- susceptible as well.

Eggplants genetically engineered with the tomato Mi-1.2 gene displayed resistance to *M. javanica* (Goggin et al., 2006).

This survey illustrates the need of controlling the *Meloidogyne* species used for germplasm screening, of using a standardized method for measuring the resistance, as well as a correct nomenclature of the *Solanum* species, in order to render comparable results obtained by different authors.

7 Insects and Mites Resistance

Eggplant is attractive to many insects and mites, such as white flies, Colorado beetle, aphids, leaf minors (*Liriomyza* sp), fruit and shoot borers, leaf hoppers, Thrips, and mites. Talekar (2003a) provides a farmer's guide to the damaging insects on eggplant in South and Southeast Asia. Apart from direct damages such as leaf parenchyma consumption and sap sucking, several pests are damaging because they are vectors of viruses or phytoplasma. Integrated pest management (IPM) based on the use of natural predators of several eggplant pests is an excellent and now commonly used alternative to genetic resistances in temperate areas and conditions of greenhouse intensive production; this method works also in some cases in the conditions of the open field, for instance for *Thrips palmi* (Nagai, 1996). IPM is strongly developed by AVRDC for controlling the eggplant fruit and shoot borer in open field in Asia (see section 7.4). However, for many insects and in particular in tropical countries, the outstanding damages of pests on eggplants are still mostly controlled by massive insecticides sprays, which are damageable to horticulturists, consumers and environment. Hence, genetic resistances constitute a valuable alternative that deserve to be investigated in depth. For some of the pests damageable to eggplant, the techniques necessary for controlling the infestation, which are a necessary pre-requisite before starting screening germplasm, exist for instance for *Thrips palmi*, for which rearing methods in greenhouse (Fernandez and Bernardo, 1999) or laboratory (Koyama and Matsui, 1992; Huang and Su, 1997), studies on populations distribution and dynamic (Bei Yawei et al., 1999), and methodology for quantifying the population (Huang and Su, 1994) exist.

The intra-specific and inter-specific diversity for pest resistance is far from being properly evaluated, mostly because of the difficulty to control the pest population, to carry out screening tests and to characterize the components of complex resistance mechanisms. However, despite the technical difficulties, the natural resistance mechanisms available in the natural diversity deserve to be investigated in depth by scientists. Robinson et al. (2001) reviewed the resistances within the eggplant related germplasm that were recorded in the literature. The engineering of transgenic resistant material is a potential alternative to natural resistances that is already strongly investigated (see section 10.4.2), but the legislation as regards to GMOs, very different from one country to another, together with various bio-safety studies

requested before release of such material, render the transgenic approach uncertain in practice.

7.1 White Flies (family Aleyrodidae, sub-order Sternorryncha, Order Hemiptera)

Malausa et al. (1988) have described cultivar differences for antixenosis (attractivity) as well as for antibiosis against the white fly *Trialeurodes vaporariorum*. Antibiotic properties affect the biotic parameters of the insect such as a reduction of females fecundity and increase of larvae mortality. However, this research did not investigate neither the morphological, nor the biochemical and genetic basis of these resistances. A simulation model combining ovi-position rate, adult survival, pre-adult survival, development period and sex ratio calculates a single criterion for estimating antibiotic resistance. This model, tested successfully on eggplant with *Trialeurodes vaporariorum* yielded better consistency results than any of the individual insect biotic parameters taken individually. It is recommended for quantifying levels of antibiotic resistance to whiteflies in breeding programs (Giessen et al., 1995). Antibiosis has also been identified among eggplant cultivars against *Bemisia tabaci* and the possible role of higher concentrations of phenols and alkaloids suggested (Soundararajan, 2003).

7.2. Aphids (Family Aphididae, sub-order Sternorryncha, Order Hemiptera)

Several aphids develop on eggplant (*Macrosiphum euphorbiae*, *Myzus persicae*, *Aphis gossypii*, and others). Apart the honeydew they produce that makes the plants sticky and allows the development of black fungus fumagine, aphids feed mostly from phloem vessels and they are vectors of several non persistent viruses such as cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), and potato virus Y (PVY). Resistance of eggplant to aphids has been poorly investigated.

The genetically engineered eggplants with the tomato gene *Mi-1.2* (which confers in tomato resistance to *Macrosiphum euphorbiae* as well as to *Meloidogyne* spp.), were susceptible to the potato aphid, thus suggesting that the aphid resistance mediated by this gene requires additional genes that are not conserved between eggplant and tomato (Goggin et al., 2006).

7.3 Leaf hopper, *Amrasca devastans* (Dist.) *biguttula biguttula* Isshida (Family Cicadellidae, sub-order Clypeorryncha = Cicadomorpha, Order Hemiptera)

These jassids, very commonly described in Indian eggplant literature, cause direct (foliage burning) as well as indirect damage by transmission of the little leaf disease. High levels of resistance, linked to a higher hairiness on leaves and affecting several biotic parameters of the nymphs and adults, have been identified within eggplant germplasm by Bindra and Mahal (1981). Schreiner (1990) confirmed this relation between resistance and dense hairiness and suggested also that green fruited varieties

suffered less hopperburn than purple fruited ones. Subbaratnam et al. (1983) found that thin leaf lamina and midribs were related to lower infestation levels. According to Lit and Bernardo (1990a), both antixenosis and antibiosis are involved in the resistance mechanisms. Lit and Bernardo (1990b) and Gaikwad et al. (1991) respectively suggest that higher alkaloids content or higher sugars, free amino-acids and polyphenols contents could also contribute to the resistance. *S. macrocarpon* and *S. viarum* were observed as highly resistant to the leaf hopper by Kumar et al. (1998). However, *S. macrocarpon* is described as only moderately resistant by Raja et al. (2001). Lower infestation rates (as well as no incidence of little leaf disease) under natural field conditions have been shown on *S. indicum* (i.e. probably *S. violaceum*), *S. insanum* (weedy type of eggplant), *S. integrifolium* and *S. gilo* as well as on several interspecific hybrids issued from these species and eggplant cultivars (Warade et al., 2004).

7.4 Fruit and Shoot Borer, *Leucinodes orbonalis* Guenee (Family Pyralidae, sub-order Heteroneura, Ditrysia: Order Lepidoptera)

This insect is described as a major pest in India and in other Asian countries; its caterpillars tunnel through shoots and fruits, thus weakening the plant and destroying the commercial value of the harvest. AVRDC published several pocket guides on the topic of the control of this pest (Talekar, 2003b; Alam et al., 2003; Rashid et al., 2003; Su et al., 2004). Talekar et al. (1999) described a procedure for rearing the pest for use in research, as well as Kumar (2004) who completes the information by providing a methodology for carrying out resistance tests; he precises that round shaped fruits are better than long shaped fruits for larvae rearing. Within eggplant germplasm, Doshi (2004) indicated that the genotypes with high phenol content, high polyphenol oxidise activity and high glycoalkaloid content, low total soluble sugars and reducing sugars, and low anthocyanin content are suitable for breeding resistant cultivars.

Resistances are described in *Solanum* species, but because of inappropriate use of species names, it is difficult to ascertain the real germplasm concerned. Behera et al. (1999) and Behera and Singh (2002) describe resistance in *S. indicum* (i.e. probably *S. violaceum*), and to lesser degree, in *S. gilo* (i.e. probably *S. aethiopicum* Gilo group), *S. incanum* (probably a wild type of eggplant) and *S. anomalum* (no suggestion for a correct species name). Kumar and Gupta (2001) show that under choice-test *S. gilo* recorded the lowest infestation percentage, and this behaviour was confirmed under field conditions. But under no choice-test conditions, all the species mentioned above plus *S. sisymbriifolium* were susceptible. Behera and Singh (2002) confirmed the interest of *S. indicum* with low infestation rate at the seedling stage and immunity for fruit infestation. Several authors report the resistance of *S. macrocarpon* (e.g. Kumar and Sadashiva, 1996), which is explained by the thick epicarp of the fruit, the large and compactly arranged mesocarp cells, and the formation of a continuous layer with lignin-like substance near the feeding zone (Srinivasappa et al., 1998; Girish et al., 2001).

Genetically engineered resistance efficient against this insect has been created (see section 10.4.2).

7.5 Colorado Beetle, *Leptinotarsa decemlineata* (Family Chrysomelidae, sub-order Heterogastra, Order Coleoptera)

No resistances have been described, though strong differences between *Solanum* species for attractivity have been observed (Daunay, pers.observ..; Robinson et al., 2001). Genetically engineered resistance efficient against this insect has been created (see section 10.4.2).

7.6 Thrips (Family Thripidae, sub-order Thripidae, Order Thysanoptera)

Thrips are vectors of the tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV), that both are able to infect eggplant (Daunay, 2007). Several species are described on eggplant, including *Thrips palmi* Karni, *Thrips tabaci* Lindeman, and *Frankliniella occidentalis* Pergande. Varietal resistance to *Thrips palmi* has been described by Yasuda and Momonoki (1988). High levels of resistance to the same thrips species have been described in wild *Solanum* species, including *S. viarum* and *S. sisymbriifolium* (Matsui et al., 1995). These species excrete sticky substances from their trichomes.

7.7 Mites (Family Tetranychidae, Order Prostigmata, sub-order Actinedida, sub-class Actinotrichida class Acari; Family Tarsonemidae, Order Prostigmata sensu lato, sub-order Tarsonemida = Heterostigmata, sub-class sub-class Actinotrichida class Acari)

Diverse mites can develop on eggplant, such as *Tetranychus* spp. -which induce foliar drying- and *Polyphagotarsonemus latus* (Bank) -leaf distortions and corky streaks on fruits-. Varietal preference has been identified for *Tetranychus ludeni* (Reddy and Baskaran, 1991), and Messiaen (1975) reports varietal differences of susceptibility to *Tetranychus* spp. Gleddie et al. (1985) report antibiosis to *T. cinnabarinus* in *S. sisymbriifolium*, as a result of the presence of toxins in its glandular trichomes. *S. macrocarpon* is said resistant to *T. urticae* by Schaff et al. (1982).

Intra-specific resistances against *Polyphagotarsonemus latus* have been described and are possibly due to thick epidermis and thick spongy leaf parenchyma: the mites, which are located mostly on the lower (abaxial) leaf face, feed with difficulties because of their not well developed mouth parts which cannot reach easily the nutritive palissadic parenchyma (Gui LianYou et al., 2001a and b).

7.8 Other Pests

Adults and larvae of the spotted beetle *Epilachna vigintioctopunctata* Fab. (Coccinellidae, Coleoptera), also known as *Henosepilachna vigintioctopunctata*, feed on the leaves, and reduce yield in the case of severe infestation. Moderate resistance levels have been found within eggplant germplasm (Raj and Kumaraswami, 1979). Rath (2005) reports resistance based on antibiosis

mechanisms. Raju et al. (1987) consider the resistance to be related to high amounts of carbohydrates and phenols in the leaves. Accessions of *S. aethiopicum* (also named *S. integrifolium* and *S. gilo*), *S. khasianum* –i.e. *S. viarum*-, and *S. microcarpum* (i.e. *S. pseudocapsicum*) were found resistant by Rajendra and Gopalan, 1998). These authors as well as Gangopadhyay et al. (1997) rated *S. macrocarpon* as moderately resistant. *S. incanum* (probably a wild eggplant) was also found moderately resistant (Gangopadhyay et al. (1997). *S. viarum* is described as resistant (absence of damages) to *E. vigintioctopunctata* as well as to *Myllocerus subfasciatus* (another Coleoptera).

8 Other Traits of Interest for Breeding Purposes

As for any crops, breeding efforts tend to cover the growers, processors, traders and consumers concerns. Hence, the cultivated material must match the local cultivation constraints and requirements, through resistance to diseases and pests as seen above, and agro-climatic adaptation for conditions as diverse as the open field in tropical or temperate areas, and heated or unheated greenhouses during various season sequences. Cultivars should also present the fruit quality requirements of each market.

8.1 Climatic Adaptation

The natural diversity available for agro-climatic adaptation is wide, covering fast to slow growth in cold or warm or hot conditions, short to long fruiting period, early to late fruiting ability, weak to vigorous vegetation, short to long internodes, erected to prostrate growth habit, etc. Root vigour, stomatal density, stomatal resistance to water vapour diffusion, have been shown to play a role in the ability of the varieties to grow and set fruits in dry or humid climates (Daunay et al., 1986). The genetic control of most of these traits is unknown.

8.2 Floral Biology and Parthenocarpy

In temperate climate, the cold conditions of winter culture under greenhouses are unfavorable to the production of a pollen of good quality and to a good pollination, and hence to a good fruit set and growth. In the 1970s and 1980s, auxins, mainly Tomatone (acide chloro-4-phenoxyacétique or 4-CPA) and Procarpil (alpha-naphtyl acetamide or NAD) spray on flowers at anthesis allowed the growers to get fruit set in such under-optimal conditions by inducing an artificial parthenocarpy. These molecules are no more in use since 2000 (in France) and the breeders have found solutions for creating good fruit setting genotypes either by breeding for a good pollen formation and the use of bumblebees in the greenhouses for ensuring the pollination, or by breeding for natural parthenocarpy which allows the formation of fruits without the need of the fecundation and seed set. Further, seedless fruits have an interest for consumers because the mass of consumable flesh is larger than in seeded fruits.

Breeders started to use natural parthenocarpy at the end of the 1980s. This trait is facultative: it is expressed only in cold conditions; as soon as the temperatures are favourable to pollination, normal fruit and seed set occurs. According to Fuzhong et al. (2005) the temperatures that induce parthenocarpy range from 7 to 10°C. Parthenocarpy is expressed quantitatively: a strong parthenocarpic tendency yields normally sized fruits containing no seeds; a weak tendency will yield undersized fruits, without seeds or with a few seeds. According to Daunay et al. (2001b) and Kuno and Yabe (2005), parthenocarpy is oligogenically and dominantly inherited. Tian ShiBing et al. (2003) found it polygenic recessive and strongly dependent on epistatic effects. The elimination or cut of the stigmas of flowers bud is an easy way for getting the expression of parthenocarpy during a breeding process (Daunay, 1981/82; Fuzhong et al., 2005). In normal pollination conditions (open field culture) the parthenocarpic material is able to undergo fecundation and hence to produce fruits containing seeds, though not always as many seeds as non parthenocarpic genotypes (Daunay, unp. results). European hybrids for greenhouse production are parthenocarpic or display a parthenocarpic tendency. Parthenocarpy has also been genetically engineered (see section 10.4.1).

8.3 Male Sterility

The interest of male sterile material is of course its use as female parent –which does not need manual or chemical emasculation- for commercial hybrid seeds production, given its male sterility is reliable, i.e. expressed whatever the agro-climatic conditions.

The first report of male sterility was that of Jasmin (1954). Phatak and Jaworski (1989) described *UGA 1-MS* line derived from a spontaneous male sterile mutant of the cultivar Florida Highbush, the functional male sterility of which was due to a failure of anther dehiscence. Phatak et al. (1991) report the monogenic recessive control of this trait (gene *fms*), and its linkage with fruit purple colour. Tian ShiBing et al. (2004) used successfully *UGA 1-MS* in breeding programs and they obtained also restoring lines, thus demonstrating the interest of this male sterility for hybrid seeds production. However, the presence of viable pollen is potentially risky if such material is intended to be used as female parent for hybrid seed production.

Isshiki and Kawajiri (2002) obtained a cytoplasmic male sterility, expressed by no dehiscence of the anthers and low pollen fertility, in the allo-plasmic backcross progenies issued from the interspecific hybrid between *S. violaceum* (female) and *S. melongena* (male). This male sterility is suggested to be the result of a disharmony between the cytoplasm of *S. violaceum* and the nucleus of *S. melongena*. Fang et al. (1985) report two other cases of cytoplasmic male sterility in back cross progenies derived from the interspecific cross between *S. gilo* (i.e. probably *S. aethiopicum* Gilo Group) and *S. melongena.* One line had petaloid anthers, and the other had vestigial, pollenless anthers, both phenotypes being stable within the range of temperatures experimented.

8.4 Fruit Colour

Fruit colour is a complex trait because it combines two pigments and their several distribution patterns (Daunay et al., 2004b). The first group of pigments, anthocyanins, has a chemical structure which comprises a nucleus, most often the delphinidol, on which various heterosides are linked (Aubert, 1971). Depending on the chemical identity and number of these additional sugar radicals, the fruit epidermis colour is perceived as more red, or more violet. These colours display various intensities, varying from the pink, to the red wine colour, to dark purple, mauve, dark violet and even black. Anthocyanins are located in the cells vacuoles of fruit epidermis. Their presence *vs*. their absence, shown by Tigchelaar et al. (1968) as oligo-genetically controlled, has been shown recently (Doganlar et al., 2002a) to be controlled by a major dominant QTL named *fap10.1* which explains 86-93% of the variation and is located on eggplant Linkage Group 10 (according to Doganlar et al., 2002a, *fap10.1* has putative orthologs in tomato). However, this apparently monogenic control is an over-simplified result given the complexity of the anthocyanins biosynthetic pathway in eggplant fruit. The also oligo-genetically controlled fruit anthocyanins intensity identified by Tigchelaar et al. (1968) has been confirmed by Doganlar et al. (2002a) who identified three QTLs.

Apart from their presence vs. their absence, and their variable intensity, eggplant fruit anthocyanins either need absolutely, need partially or do not need at all the presence of light to be synthesized in the fruit epidermis (Figure 1 left). Phenotypically, when the anthocyanins need absolutely light to be synthesized, the fruit skin under the calyx (not exposed to light) is completely anthocyanins less, and the rest of the fruit can be regularly or irregularly purplish or violettish. If the anthocyanin are only partially dependent on light or light independent, then the fruit skin colour under the calyx is only clearer than on the rest of the fruit or displays the same intensity. The presence of more or less anthocyanins under the calyx is mono-genetically controlled by the dominant gene *Puc -purple under calyx-* (Tigchelaar et al. 1968); conversely, the absence of anthocyanins under the calyx (allele Puc^{+}) is monogenic recessive. The involvement of some photoregulated enzymes of the anthocyanins biochemical pathway such as phenylalanine ammonia-lyase -PAL- and chalcone synthase -CHS- (Winkel-Shirley 2002) and/or the involvement of transcription factors regulating the expression of the genes encoding these enzymes (Takos et al., 2006) should be investigated for unravelling the molecular control of this trait in eggplant fruit. Anthocyanins distribution, apart from uniform or irregular and susceptible to light, can also be striped (Figure 1 right)

The second group of pigments, chlorophylls A and B, mostly located in the fruit sub epidermal cell layers are responsible of the green colour of the flesh as well as of the epidermis. The green colour can be masked by anthocyanins if they are also present. The presence *vs* the absence of chlorophylls has been demonstrated as controlled by a single dominant gene named *G* -for Green- by Tigchelaar et al. (1968). The various nuances of green (clear yellowish green to dark green) observed among green fruited varieties are not genetically explained yet. Chlorophylls distribution displays two main patterns: uniformity or reticulation (Figure 2). In the case of reticulation, the upper part of the fruit (calyx side) is dark green, and this

colour ends up in elongated and irregular patterns when reaching the bottom part of the fruit (blossom scar side) which is light green. The irregular distribution pattern (Figure 2) has been shown by Tigchelaar et al. (1968) to be mono-genetically and dominantly inherited (gene *Gv* -for Green variegated-) and Doganlar et al. (2002a) identified a QTL on LG4 (*fst4.1*) explaining 49-67% of the phenotypic variation that probably corresponds to *Gv*. According to Doganlar et al., 2002a, *fst4.1* has putative orthologs in tomato.

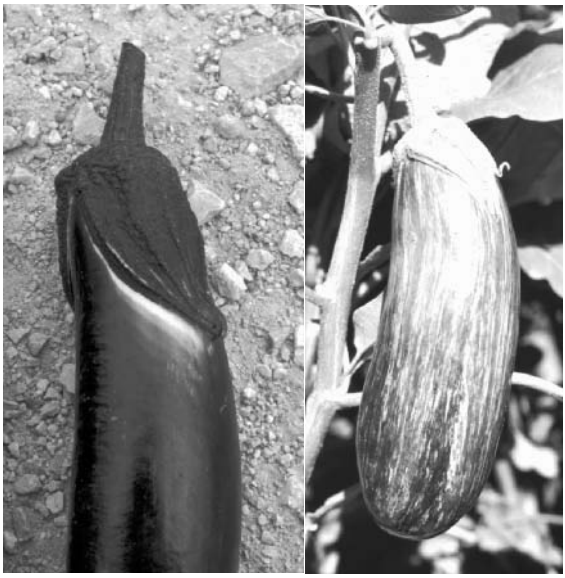

Fig. 1. Fruit color in eggplant: anthocyanins susceptible to light (left), and anthocyanins striped (right). In both cases the green background is due to the presence of chlorophylls.

Fig. 2. Uniform and variegated (reticulated) distribution of the chlorophylls in eggplant fruit.

The combination of the presence of anthocyanins and/or chlorophylls, with various combinations of their distribution patterns, is responsible for the great color diversity found in eggplant (Figure 3). At physiological maturity, epidermis color turns to bright yellow (for initially white or green fruits) or to a brown (for initially violet fruits).

Fig. 3. Fruit color diversity (yellow and brown fruits are physiologically mature, the others are immature).

8.5 Fruit Bitterness

Eggplant specific piquant and bitter taste is due to the presence of two kinds of steroidic saponosides, which chemistry is quite complex (Aubert et al., 1989a). The first kind belongs to the glyco-alkaloids chemical category; these compounds have a steroidic nucleus to which a nitrogenic heterocycle as well as heterosides (sugar radicals) are attached. The dominant glycol-alkaloid specific to eggplant is solasonine, the main nitrogenic heterocycle (or aglycone) of which is solasodine. Solasonine is the eggplant analogous of the potato solanine (aglycone solanidine) and the tomato tomatine (aglycone tomatidine). The second kind of steroidic saponosides relates to melongosides with a furostanol or spirostanol type structure. These compounds are composed by a non nitrogenic heterocycle as well as heterosides which are attached to a steroidic nucleus.

All these steroidic saponosides are physiologically active (Aubert, 2002) and have in particular anti-microbial, hypocholesterolemic, anticancerous, and

hypotensive actions that have of course an interest in preventive nutrition, and which have been used for ages in traditional Indian medicine (Khan, 1979a). Their lipophily explains eggplant affinity for oil during its culinary preparation. Solasonine and melongosides are bitter compounds and the higher their concentration in the fruit, the bitter its taste. Their potential toxicity at high concentration is associated with unpalatability.

Eggplant fruit bitterness depends on varieties, but cultivation and harvest methods have a strong influence: the older the culture, the more pathogenic or abiotic stresses on it, the more mature the fruits harvested, the bitter the fruits (Aubert et al., 1989b). Depending on the markets and also on the consumers, some bitterness or the absence of bitterness is requested and thus bitterness should not be systematically eliminated from the breeding material because the consumer demand is diverse. Objective, easy, fast and reliable measurement methods of bitterness are not available yet, but this technical limitation should find sooner or later a solution, which would easy the evaluation of the breeding material for this trait. The inheritance pattern of this important part of the fruit quality is not known so far, but research work is ongoing (Doganlar et al., unpub. results).

8.6 Fruit Phenolic Compounds

The anthocyanins (phenols group of flavonoids) of the fruit epidermis as well as the phenolic acids (mostly hydroxycinnamic conjugates, with chlorogenic acid as predominant compound) present in the flesh have anti-oxidant properties interesting from the nutritional and sanitary standpoints, since they are very effective free radicals scavengers, and hence they contribute to reduce radicals-mediated pathogenesis such as carcinogenesis and atherosclerosis (Stommel and Whitaker, 2003). They have also a hypo-lipidemic as well as an anti-microbial action. These substances are the substrates for the polyphenol oxidase enzyme which activity leads to the rapid browning of cut or injured tissues, this having a negative impact on the fruit flesh and skin aspect. They also contribute to the fruit organoleptic properties since they generally impart a bitter taste and interfere with other molecules during the cooking process.

Important cultivars differences (up to 20 fold range) for the phenolic acid content and flesh browning rapidity have been demonstrated by Stommel and Whitaker (2003) and Prohens et al. (2007). This variability offers the breeders the possibility to carry out a selection either for increasing the phenolic acids content, thus improving the health value of the fruit but depreciating the aesthetic value because of a more intense and rapid flesh browning after cut, or oppositely, for decreasing it and thus reducing the health value but improving the aesthetic value through a lesser flesh browning. This unsatisfying alternative could possibly be turned round by other potential solutions that could be investigated for increasing the health value and decreasing the flesh browning after cut. Theoretically, a workable solution could be for instance a breeding for high phenolic acids content and low concentration or down regulation of the enzyme responsible of the browning (polyphenol oxidase), or a breeding for a low phenolic acids content in the flesh and a high anthocyanins content in the epidermis. The genetic control of the flesh phenolic acid content and

of the polyphenol-oxidase bio-synthetic pathway is not known yet, but work is in progress (Prohens et al. unpub. results; Doganlar et al., unpub. results).

8.7 Prickliness and Hairiness

Prickliness is important for growers, traders and consumers, since prickles can damage fruit epidermis when the fruits are handled during and after harvest, and the prickles are also aggressive towards the workers's hands that handle plants and fruits. Hence, the absence of prickles is generally considered as a desirable trait, but in some areas such as Nagpur in central India, the presence of prickles on the calyx is considered to be desirable because consumers consider it to be linked to a good fruit organoleptic quality. Prickles presence on leaves, stem, petioles and fruit calyx has been shown to be associated to a major QTL located on chromosome 6 (Doganlar et al. 2002a).

Hairiness is another important trait since the stellate hairs of eggplant cause a stinging sensation on skin and adversly affects the respiratory system of people who handle the plants. Large cultivar differences exist for this trait, from very hairy to almost glabrous. The genetic control of hair absence was suggested to be monogenic recessive and affected by modifier genes (Daunay et al. 2001b) but Frary et al. (2003) revealed the quantitative nature of the inheritance.

8.8 Other Traits

Chadha (1993) surveyed the inheritance pattern of many morphological traits relative to plant (e.g. height, spread), anthocyanins presence on diverse organs (e.g. hypocotyl, leaf, stem, flower, fruit), fruits (e.g. girth, weight, shape, number, number per cluster), some being monogenetically controlled and others quantitatively inherited with high or low heritabilities. Molecular mapping of the genetic factors controlling such traits has started recently (see section 10.5) and brings new insights. For instance, 4 QTLs contribute to plant height (Frary et al., 2003), many QTLs contribute to anthocyanin intensity on many plant organs and several co-locate on LG6 and LG10 (Doganlar et al., 2002a), 3 QTLs contribute to fruit weight and 3 others to fruit shape (Doganlar et al., 2002a), 4 QTLs contribute to the number of fruits per infructescence (Frary et al., 2003) and so on. These authors found also that many of these QTLs have counterparts in other Solanaceae, in particular tomato. This type of molecular analysis will also bring new insights at the additive, dominant and epistatic effects involved in many eggplant morphological traits.

9 Reproductive Biology, Breeding and Creation of cultivars

9.1 Reproductive Biology

Eggplant is a diploid species ($2n$=24), non strictly autogamous. Depending on the climatic conditions and on the presence of insects such as bumble bees, the outcrossing rate can vary widely from zero to above 70% (warm conditions and

presence of pollinators). Eggplant does not suffer inbreeding. F_1 hybrids exhibit yield heterosis, as well as a larger homeostasis (relative stability of the performances) than pure lines as soon as the agro-climatic conditions shift from optimal. The lesser genotype x environment interaction for heterozygous material (compared to homozygous) has been demonstrated by Manicacci (1988) quoted by Daunay (1987-1988). The F_1 genetic structure is also a method for cumulating complementary parental traits, in particular disease resistances, as well as for protecting the creative work of breeders.

9.2 Breeding Context

In the public sector, research on eggplant is quite active, through the involvement of several but mostly small groups of scientists in particular in Europe, USA, Turkey, and Japan, and especially India, the homeland of eggplant. In the private sector, the Europeanization of the seed market in the 1980s, followed by its globalization at the world level from the 1990s, have boosted the interest of the vegetables seed companies for this species, which is widely cultivated in temperate areas and even more widely cultivated under the tropics, with a hotspot in Asia.

For 30 years, breeders have created new eggplant types, in particular those adapted to winter production under greenhouses (reduced vegetation, good fruit set, and several fruit quality traits) for various Mediterranean production areas and production calendars. Intra-specific variation for developmental traits such as the number of leaves before the first inflorescence, the internodes length, the growth speed, the growth habit, the root vigour, and the ability to produce fruits over a long production period, has been insufficiently evaluated and used by breeders, and a more systematic characterization of the germplasm for those traits would undoubtedly reveal useful natural variation for breeding.

9.3. Breeding Methodologies

Breeding methodologies applied to eggplant have emphasized pedigree selection as well as back crossing methods which are well adapted to autogamous plants and traits of simple inheritance (monogenic). However, these methods have a low creative potential since they are applied to the narrow genetic basis of a few parents. The need to create cultivars with whole sets of improved traits for new production conditions (e.g. greenhouses round the year), new markets, the even sharper and specific market requirements (concerning fruit shape, color, firmness, homogeneity, conservation), and disease resistances, has boosted the search on breeding methodologies. The interest of the recurrent selection for autogamous species (this method was first set up on allogamous species) has been explained by Pochard (1978). This method is based on the use of a wide genetic basis where all the traits desired are present, but dispersed among the genotypes constituting the starting population. Through several cycles of inter-crossing the best performing families and genotypes are selected and inter-crossed. The inter-crossing allows the progressive recombination of many desired traits, as well as the obtention of transgressive material (with phenotypes covering a wider range of values than the parents) for

quantitatively inherited traits. If correctly carried out, this method is extremely creative since it yields the recombination within single genotypes of genes and alleles formely dispersed among many genotypes. Palloix et al. (1990a and b) achieved outstanding results by using this method for breeding another autogamous Solanaceae, capsicum pepper, resistant to *Verticillium* wilt and *Phytophthora capsici*. Thabuis et al. (2004) analyzed the molecular basis of these breeding achievements. Recurrent selection was also successfully applied to eggplant for creating genetic material of good agronomic characteristics for the open field and greenhouse; however, the results demonstrated also indirectly the importance of optimizing the choice of the genotypes constituting the first population, for obtaining favourable recombinations along several successive cycles (Daunay 1979-1980 and 1981-1982). Indeed, if the genetic basis of the first population is too narrow, the progresses stop rapidly after a few generations of intercrossing.

At the end of a recurrent selection program, the return to homozygous material is carried out either by selfing for at least 6 generations, or better, by anther culture (see 10.1) which produce within 2 years pure lines (100% homozygous)

9.4 Cultivars

In Europe, seed companies concentrate their breeding efforts on cultivars aimed at being cultivated in the mass production areas. The successfully commercialized cultivars have morphological and developmental features specific to each of these areas, their culture types and production calendars. The eggplant seed market is said conservative, i.e. when a cultivar is adopted by growers and traders, it is difficult to replace it even with a better performing one. This means that the longevity of a variety can be quite long (10 years or even more) if and once it has succeeded in some production area.

The European catalogue, which is a good image of the breeding efforts, included at the time of its creation for eggplant (1970) only populations and lines. The first hybrids were registered shortly after 1970, and their percentage over conventional cultivars increased progressively along the time: it was 67% in 2000 and 72% in 2005 for a total of 247 cultivars. 21 new cultivars were registered in 2005. These data reflect a moderate breeding activity on eggplant, which is much less than for tomato for which in 2005 the catalogue counted 2404 cultivars with 83% F1 hybrids!

Eggplant F_1 hybrids are common in Japan for a long time; indeed some 20 hybrid cultivars were already commonly used in the 1935s (Hallard, 1996). Hybrids are developing rapidly in other Asian countries, such as India and China, where the genetic erosion of traditional varieties is hence speeding up. In Europe, though having suffered considerable genetic erosion since the 1960s, traditional cultivars are still cultivated and commercialized locally at a small scale, mostly in South European countries such as Spain and Italy. Those cultivars have original traits, such as round fasciated shapes (e.g. the Italian Violetta di Firenze type) or white and purple striped epidermis (e.g. the Spanish Listada de Gandia type). For some years, there is an increasing and renewed interest for such varieties of consumers, Cook chefs together with alternative growers who privilege sustainable production methods, the symbolic and gustative quality of traditional varieties and direct

commercial links with the consumers. If this tendency persists, the profile of eggplant production in Europe will tend to the co-existence of a delocalized mass production for mass consumption markets, together with a local production for more fortunate consumers.

Eggplant cultivars types and diversity differ from one country to another one. European and American commercial cultivars are characterized by fruits of 200-400 g, globose, intermediate or long, with an almost black and shiny epidermis and a green almost unarmed calyx (Figure 4). South European traditional cultivars (landraces) are much more diverse, in particular for the fruit color (white, white striped with purple, reddish, violet and white, purple and green). In Sudan, the Black Beauty type is dominant (large, globose, slightly fasciated, and dark purplish fruits). In Thailand one finds much smaller fruits of diverse colors, such as green ping pong shaped and sized fruits, together with small long violet or green or white fruits. In China, there is a great diversity of fruits types, for instance large fruits of round, intermediate or long shapes, and of a violettish color influenced by light (shaded parts of the fruits have a lighter color than the ones exposed to light, or they have even no violettish color at all). Japanese dominant fruit types, harvested very young, are characteristically very shiny, almost black, and quite soft when taken in hand. But that is in India where one finds the greatest diversity of fruit sizes, shapes and colors.

Fig. 4. Commercial types of eggplant in Europe: long, intermediate and globose. These fruits have a black and shiny skin, with a green calyx.

10 Biotechnologies: In Vitro and DNA Technologies

The use of biotechnologies combined with conventional breeding methods is the key to future improvement of eggplant. Biotechnological methods have been developed on eggplant for about a quarter of a century, and they are presently widely used in eggplant breeding processes. Eggplant tissues are easily grown in vitro, thus micro-propagation, somatic embryogenesis, protoplast culture and protoplast hybridization, plant regeneration from cells or tissues, and anther culture are carried out successfully. Genetic engineering (transgenesis) is carried out since the 1980s and molecular mapping is developing since the end of the 1990s. For a review, see Collonnier et al. (2001b), and Kashyap et al. (2003). We will concentrate here on the techniques and results intended at interfacing directly with breeding programs.

10.1 Anther Culture

In vitro androgenesis has been successfully applied and adapted to eggplant by Dumas de Vaulx and Chambonnet (1982). In addition to the rapidity of this method for yielding homozygous material, it has other important advantages such as the simplification of the analysis of traits genetic inheritance, as well as the increased probability to obtain multi-recessive genotypes. This technique is routinely used since the end of the eighties by seed companies carrying out eggplant breeding programs.

10.2 Somatic Hybridization

This method is based on the in vitro fusion of somatic cells (generally diploids cells of the leaf mesophyll of two plant partners), followed by the regeneration of plants from the fusion product. It has been successfully applied to eggplant since the 1980s for obtaining interspecific hybrids, in particular with species not sexually crossable with eggplant. The first interspecific somatic hybrids were obtained between eggplant and *S. sisymbriifolium*, which is, as seen in the section 6, a promising source of diseases resistances. Using the PEG (Poly Ethylen Glycol) as a fusion agent, Gleddie et al. (1986) obtained aneuploids somatic hybrids, which displayed developmental abnormalities as well as total sterility. Later on, tetraploid hybrids from this somatic cross were obtained by using electro-fusion (Collonnier et al., 2003), but the hybrids were still sterile. Hence, somatic hybridization brought this particular interspecific cross once step further than the sexual cross (which did not yield a viable hybrid), but in terms of application to breeding purposes, this technique failed so far.

Somatic interspecific hybrids were also obtained by using *S. torvum* (another promising source for disease resistances) as fusion partner of *S. melongena*: first by Guri and Sink (1988a) with PEG, and by Sihachakr et al. (1989, 1994) with electro-fusion. As their sexual counterparts, the tetraploid somatic hybrids did not produce progenies because of their sterility. However, a limited gene transfer thanks to the asymetric fusion between *S. melongena* protoplasts and X-ray irradiated protoplasts

of *S. torvum* yielded fertile somatic hybrids, diploid or tetraploid, which carried the *Verticillium* tolerance of *S. torvum* (Jarl et al., 1999).

Somatic hybridization also succeeded with *S. viarum* -known as *S. khasianum* in India- (Sihachakr et al., 1994) but the somatic hybrids (tetraploid or aneuploid, depending on the hybrids) though displaying 12% of stainable pollen produced parthenocarpic fruits, and hence were as sterile as their sexual counterparts (see Table 2).

In the case of *S. aethiopicum* Aculeatum and Gilo Groups, tetraploid and partially fertile somatic hybrids were obtained (Daunay et al., 1993), which yielded di-haploid progenies via anther culture (Rizza et al., 2001). However, as for this particular cross the sexual path is also workable (Table 2, Ano et al., 1991), the somatic hybridization in this case again did not bring a decisive advantage over the sexual hybridization.

The applications of interspecific somatic hybridization to eggplant and its wild relatives did not bring so far substantial progresses (when compared to sexual hybridization) for transferring genes of interest into eggplant genome. However, as the electro-fusion technique works well for yielding well balanced tetraploid somatic hybrids, and as there are many more *Solanum* species that would be worth to be used in somatic fusion experiments (those which did not yield so far fertile progenies when crossed with eggplant), one can expect that sooner of later, somatic hybridization will prove itself as a precious method for transferring genes from wild germplam into *S. melongena* genome. The return to the diploid level is (relatively) easily obtained by using anther culture (Rizza et al., 2001; Rotino et al., 2005). The scientists who use this technique should however use several accessions of each parental species, instead of only one combination of parental accessions as mostly done. Indeed, the sexual hybridizations have shown that the success of a given interspecific cross depends sometimes on the parental accessions used (Daunay et al., 1999), and this case could also happen for somatic interspecific crosses. Besides, this influence of the intraspecific diversity on the success of interspecific crosses in *Solanum* species raises interesting questions as regards to the mechanisms involved in interspecific crossing barriers.

More distant hybridizations using eggplant as one of the fusion partners, interspecific (with *Solanum nigrum*) or intergeneric (with *Nicotiana tabacum*, and *Lycopersicon* spp.) have been carried out, with various successes that have mostly an academic interest (Collonnier et al., 2001b).

10.3 Somaclonal Variation

Resistant cells and derived plantlets were obtained from cell suspensions exposed in a liquid medium to culture filtrate of *Verticillium dahliae* ((Rotino et al., 1987). The potential of somaclonal variation induced by auxins applied to cells culture undergoing somatic embryogenesis has been investigated by Hitomi et al. (1998).

10.4 Genetic Engineering

As eggplant is easily manipulated in vitro, genetic engineering via *Agrobacterium tumefasciens* vector was successfully applied to it from the 80s (Guri and Sink, 1988b; Fari et al., 1995), but the technique applied to eggplant is still the subject of researches (e.g. Pessarakli and Dris, 2003; Picoli et al., 2002; Franklin and Sita, 2003; Magioli and Mansur, 2005; Kumar and Rajam, 2005). The genetic transformation carried out so far concern mostly parthenocarpy, resistance to insects and abiotic stresses. We provide hereafter an overview of the achievements.

10.4.1 Parthenocarpy

Though existing in the natural genetic diversity of eggplant germplasm, parthenocarpy has been genetically engineered (Rotino et al., 1997) by using a chimaeric parthenocarpic gene (*DefH9-iaaM)* associating (1) the placental and ovule specific promoter *DefH9* from *Anthirrinum majus*, and (2) the *iaaM* gene from *Pseudomonas syringae* pv. Savastanoi which codes for a tryptophane monoxigenase which is converted into indol-3-acetic acid. The expression of the chimeric gene induces parthenocarpic fruit development. Donzella et al. (2000) and Acciarri et al. (2001) showed that the productivity of the transgenic material in grrenhouses and open field was higher that that of the non transformed control, even when this latter was treated with phyto-hormones inducing parthenocarpy. Though technically successful, such transgenic material could not be released on the seed market, given the reluctance of the European market to genetically modified organisms (GMOs), and thus this parthenocarpy is not used in practice.

10.4.2 Insects and Nematodes Resistance

Resistance to Colorado beetle (*Leptinotarsa decemlineata*), one of eggplant pests against which no eggplant germplasm resistance is described, has been successfully engineered by using a *Bt* gene encoding for the CryIIIB toxin (Arpaia et al., 1997). Field experiments with natural Colorado beetle infestation demonstrated the effectiveness of the resistance (Arpaia et al., 1998): transgenic plants suffer a lesser infestation level than the control, and hence produce a better yield. The observations on the experimental crop did not detect any deleterious effect on other insects, thus confirming the specificity of the CryIIIB toxin expressed by the transgenic plants. The technical success of this genetic transformation could not be transferred into practice, for the same reasons as the transgenic parthenocarpic material.

Resistance to *Leucinodes orbonalis* (eggplant fruit borer, a very serious problem on eggplant in Asia, as seen in section 7.4) was engineered with a synthetic *cry1Ab* gene coding for an insecticidal crystal protein of *Bacillus thuringiensis* (Kumar et al. 1998), and the transgenic material had a significant insecticidal activity against the insect larvaes. Next researches have led to the creation of genetic material carrying the *cry1Ac* gene associated to the CaMW35S promotor, and displaying an insecticidal activity (about 100% of larvae mortality) efficient against *Leucinodes orbonalis* and also *Helicoverpa armigera*. This material is engaged since the

beginning of the 2000s in many biosafety investigations for obtaining the authorization to be commercialized, and agronomic managing strategies of the transgenic resistance are under study (J.L. Karihaloo, pers. commun.).

Resistance to *Myzus persicae* (peach tree aphid) and *Macrosiphum euphorbiae* (potato aphid) has been also engineered (Ribeiro et al., 2006) by incorporating the rice oryzacystatin gene, which encodes for a cystatin (inhibitory protein) targeting digestive cystein proteases of pests such as coleopteran insects and nematodes. The transgenic eggplant reduced several biotic parameters of the target insects (*Myzus persicae* and *Macrosiphum euphorbiae*) such as the reproductive rate and life longevity. However the transformation of eggplant for this gene did not confer resistance to *Meloidogyne* spp. (Ribeiro et al. (2004). Eggplants were also transformed for *Mi-1.2* tomato gene, which confers resistance to root-knot nematodes, *Macrosiphum euphorbiae* and *Bemisia tabaci* (Goggin et al., 2006). The transgenics expressed resistance to *Meloidogyne javanica* but were fully susceptible to *Macrosiphum euphorbiae*. This experiment demonstrates that *Mi-1.2* can confer nematode resistance to eggplant, but indicates also that the requirements for Mi-mediated aphid and nematode resistance differ between tomato and eggplant.

10.4.3. Resistance to Abiotic Stresses

The mannitol-1-phosphodehydrogenase (*mtlD*) gene introduced into eggplant by Prabhavati et al. (2002) conferred tolerance against osmotic stresses induced by salt, drought and chilling treatments.

10.5 Molecular Mapping, Markers and Marker Assisted Selection

Genetic mapping in eggplant has been initiated in the last decade only. The first molecular map was developed on an intraspecific F_2 population with RAPD markers defining 13 linkage groups (Nunome et al., 1998). This map was completed with AFLPs (Nunome et al. 2001) and SSR (Nunome et al., 2003b) and contains on the whole 162 markers in 17 LGs encompassing 716,9 cM at an average spacing of 4,9 cM. Doganlar et al. (2002b) developed a map from an interspecific F2 population (*Solanum linnaeanum* x *S. melongena*) (Figure 5), with 12 LGs, 233 RFLP markers – previously mapped in tomato- and spanning 1480 cM. This map, comparable to the tomato map, showed colinearity between several LGs of the two species, and 5 translocations and 23 paracentric inversions for the rest of the LGs. The strong conservation between eggplant and tomato chromosomes suggest that the genomic resources developed for tomato will be useful for eggplant genetic research. Another interspecific map from the same interspecific cross was developed by Sunseri et al. (2003) with 273 AFLP and RAPD markers encompassing 736 cM in 12 LGs. Current progress is being made for saturating these maps with the addition of other PCR-based markers, in particular SSR (Nunome et al., 2007) and AFLPs (Frary et al., unpub.) and for developing common markers allowing the comparisons between maps.

Fig. 5. Mapping F_2 population *S. linneanum* MM 195 x *S. melongena* MM738, used by Doganlar et al. (2002a and b) and Frary et al. (2003). Parents are situated in the top of the picture (*S. linneanum* to the left; *S. melongena* to the right).

Genes and QTLs mapping for horticultural traits is running full speed since the establishment of the first maps, but so long the different existing maps are not anchored to each other, the comparison of the genes and QTLs locations obtained on the one or the other is very difficult. Among the traits focused to for mapping purposes, one finds *Verticillium* wilt resistance (Sunseri et al., 2003), bacterial wilt resistance, fruit shape and color, stem and calyx violet color (Nunome et al., 1998, 2001), and a wide range of characteristics (number, shape, size, violet color, etc.) of many plants organs (flower, ovary, fruit, leaf, prickles, hairs) (Doganlar et al., 2002a; Frary et al., 2003). These latter authors found that the domestication of eggplant, like that of other plants, involved mutations at a limited number of loci; they identified also several loci which have putative orthologs in tomato, potato and pepper, for fruit weight and shape, fruit color, leaf shape and size, flowering time and flower number, plant height, and hairs. Marker assisted selection of eggplant has been limited so far, but the ongoing genetic studies will provide within a short time the convenient tools for implementing it into the breeding programs. Genomic resources for eggplant are developed actively such as ESTs (Solanaceae Genomics Network (http://www.sgn.cornell.edu); Fukuoka et al., 2006) and BAC libraries (Frary et al., 2007). Further, the current international effort to sequence the tomato genome will provide a wealth of information applicable to eggplant.

11 Conclusion

Eggplant is a crop of a quite fascinating history and biology. Though concentrating less scientific efforts than other Solanaceae, a wide range of worldwide research applies to it as attested by the scientific literature. Given its close genetic similarities with tomato, it is in position to benefit directly the powerful genomic tools developed on this sister Solanaceae, in particular for identifying and mapping the genetic factors involved in its agronomic traits, and for investigating at the molecular level their allelic diversity. Conversely, eggplant specificities can interfere with research on and breeding of other Solanaceae, for instance the wide range of its close wild relatives is a potential reservoir of new rootstocks to be used (at least for eggplant and tomato), and its lesser general susceptibility to viruses is a potential (but probably difficult) field of investigation for the interaction of Solanaceae with viruses and their vectors.

Eggplant intra and interspecific genetic resources are still far from being properly characterized for many traits of interest, but the results obtained so far in the field of the evaluation for pest and disease resistance show how useful they already were and how promising they still are. It is in the interest of all scientists and breeders to strengthen their international collaborations in this particular field. Indeed, the survey of the pests and disease resistances in the wild germplasm, though not exhaustively carried out here, demonstrate that the resistant or susceptible behaviour depends not only on the *Solanum* species, but also on the accessions, on the pathogens strains or races or pathotypes, as well as on the type of symptoms recorded. The wealth of *Solanum* species, together with the frequent use of inaccurate plant species names complicate furthermore the comparison of the results obtained by different authors as well as the identification of the material really worth to be used in breeding programs. Concerning the use of a correct nomenclature for designating the species, Daunay et al. (2006), provide web site addresses where taxonomic information can be found (this document is downloadable from http://www.ecpgr.cgiar.org/Workgroups/solanaceae/solanaceae.htm).

The interspecific crosses having succeeded so far (Table 2) demonstrate the wide genetic basis that eggplant breeders can already characterize and use. However, breeders should keep in mind that unexpected and undesirable traits can be also and unintentionally transmitted through interspecific hybridization, such as a particular susceptibility (e.g. the susceptibility of *S. linnaeanum* to PVY, or the susceptibility of *S. torvum* to fruit anthracnosis). Therefore, any particular known undesirable trait in a given *Solanum* species should be systematically looked at and eliminated from its interspefic progenies.

The in vitro cellular (somatic hybridization) and genetic (transgenesis) bio-technologies are an important resource for the breeders. Sometimes applied to agronomic problems that have solutions by the use of the natural genetic diversity and common breeding techniques, their use should be better concentrated on *Solanum* species and/or traits for which conventional resources are seemingly helpless, such as the resistance to major pests in tropical countries. However, the advantages provided by the use of transgenic material are seriously balanced with biosafety potential dangers, in particular gene escape by pollen flows to other

cultivars and wild relatives growing in their vicinity. This risk is maximum in India and the Asian countries where spontaneous wild and weedy forms of *S. melongena* exist, as well (though to a lesser extent) as in Middle East and African areas where the relatively easily cross-compatible *S. incanum* groups C and D grow spontaneously. Thus, the concerns over the control of disastrous insect damages on a very popular crop, versus the risk of transgene escape with unknown and uncontrolled consequences must be considered.

Finally for eggplant (as for any crop), genetics should not be considered as the unique resource for solving agronomic problems, and a closer collaboration between agronomists, IPM managers, and breeders in certainly one of the present challenges scientists and breeders face or will face in a near future, in the context of a food production respecting sustainable horticultural practices.

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Pepper

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1 Introduction

Cultivated peppers are all members of the new world genus *Capsicum*. Their production and consumption have steadily increased in the United States and worldwide during the 20th century due to their roles as both vegetable and spice. Just like their solanaceous cousins, tomatoes and potatoes, peppers have rapidly become an important component of diverse cuisines around the world. This is reflected in the large acreages devoted to their production in such countries as India, Mexico, China, Korea, and the United States (Table 1). In addition, interest in both sweet and pungent types of peppers is growing in many countries not traditionally associated with spicy cuisine. Protected culture has developed in northern latitude countries such as Holland and Canada and also in Mediterranean countries such as Spain and Israel, to supply the increased demand (Shaw and Cantliffe, 2003). Much of the recent attention focused on peppers can be attributed to their unique pungency, which has made them an important spice in the cuisines of various countries. The proliferation of ethnic restaurants and food products from such countries as Mexico, India and Thailand has positively influenced the demand for peppers throughout the world. Both sweet and hot peppers are processed into many types of sauces, pickles, relishes and canned products. Consumption of these condiments and snack items has surpassed tomato based condiments, such as ketchup, in the United States. Exploitation of the unique flavours of processed peppers, such as chipotle and Habanero, by the fast-food industry has also led to burgeoning demand for hot peppers in urban areas worldwide. This has led to extensive production of hot peppers in some countries for export markets. A substantial percentage of pepper acreage in the largest producing countries is dedicated to chilli powder. However, the higher prices received by farmers for fresh product have helped sustain the vegetable pepper industry, despite rising production costs. Competition and increased demand

have led to improved production practices and higher quality fresh peppers in the marketplace. This has been a positive development for plant breeders, as demand grows for improved varieties of diverse fruit types.

Table 1. Pepper cultivated hectares for major producing countries (Source: TAES, 1993).

Country	Acreage (ha)
India	902,449
Ethiopia	246,049
Indonesia	218,935
Korea	133,915
China	87,007
Mexico	82,556
Bangladesh	79,723
Nigeria	78,104
Thailand	61,108
Pakistan	58,275
USA	50,586

In addition to the importance as a food, peppers have also received attention recently for their potential as nutraceuticals. This is due to their high levels of phytochemicals with documented human health benefits. These include carotenoids, flavonoids, ascorbic acid, phenolic compounds and the pungent capsaicinoids (Bosland and Votava, 2000; Crosby 2005c). The benefits of these compounds, as powerful anti-oxidants, have been documented in numerous crops, while capsaicin has recently been implicated in prevention of certain types of cancerous tumours (Ito et al., 2004). The pungent compounds are also utilized extensively in the pharmaceutical industry for their analgesic properties (Drugs.com, 2006). Natural and synthetic forms of capsaicin are also being utilized by the law-enforcement and self-defence industries as relatively safe alternatives to other chemical, electric or physical restraint mechanisms. These are also being utilized for other industrial purposes, such as the deterrence of various pests in crops, wood products and plastic cable coatings (Scorecard, 2006; Machinedesign.com, 2000).

Beyond the realm of commercial production there also exist a large number of pepper aficionados among home gardeners, hobbyists and even artisans. The relatively small space required to cultivate pepper plants has made them popular with backyard and container garden enthusiasts. Numerous ornamental types have been developed to add landscape appeal to many yards and formal gardens. In the southwest region of the United States, dried long chiles are used to create wreaths and other decorative works of art. The fascination with hot pepper sauces, chilli and spicy cuisine has influenced urban and rural culture in the US and elsewhere. Festivals, cook-outs and salsa competitions have become important cultural events, attracting people in greater numbers each year. In this regard, peppers may have the greatest impact on bringing diverse cultures together of any vegetable.

2 Origin and Domestication

Extensive evidence collected by botanists and historians has created a reasonably clear picture of pepper origins and distribution. The word 'Capsicum' was first used to describe the genus by Turnefourt in 1719, and later adopted by Linnaeus (Andrews 1984). Prior to that, much confusion existed about the botany of peppers, which were sometimes confused with black pepper, *Piper spp*., or other well-known spices. All species of the genus *Capsicum* originated in the new world tropics. Mexico is the centre of diversity for *Capsicum annuum L*., the most important cultivated species. Wild forms of this species are also found from Texas south to Argentina. The Amazonian regions of South America comprise the centre of diversity for *C. chinense* and *C. frutescens,* with secondary centres in Central America and the Caribbean islands. These three cultivated species are part of the Annuum complex along with the possible progenitor species, *C. chacoense* (Bosland and Votava, 2000). *C. baccatum* diversity is greatest in low to mid elevation regions of Bolivia, Peru and Brazil, south of the Amazon basin. The Baccatum complex includes *C. praetermissium* and *C. tovarii*, as possible wild progenitors. The mid-elevation, Andean regions of Bolivia and Peru are the primary centres of diversity for *C. pubescens*. Its species complex includes *C. eximium* and *C. cardenasii* (Bosland and Votava, 2000). Extensive diversity for *C. baccatum* also exists in western regions of Brazil and Colombia (Andrews, 1984). The remaining wild species are scattered throughout South America, and even as far abroad as the Galapagos islands in the case of *C. galapagoense.*

Domestication of the different species likely occurred over a period of thousands of years by the pre-Columbian cultures of the western hemisphere. These Native Americans domesticated corn from teosinte in Mexico about 8000 years ago, and it is likely that peppers may have also been utilized by the same people. The process of selection and landrace development likely occurred subsequently over thousands of years. Pickersgill (1969) suggests that this process may have begun 4000 years ago. Remains of peppers in Mexican archaeological sites dating back to 7000 B.C., may suggest even earlier domestication by Native Americans (Andrews, 1984). The diverse fruit and plant types within the species *C. annuum*, point to extensive human selection by multiple groups over many generations. Though less variation exists in fruit types of the other cultivated species, it is clear that human selection also occurred in various regions for specific characteristics as well. Even in the last hundred years, much more attention has been given to breeding and selection within *C. annuum* than the other species. This probably is the result of more extensive distribution of this species by the early European explorers throughout the world (Andrews, 1984). The potential for larger sized fruit may also be a reason why *C. annuum* has remained the most important cultivated species.

3 Varietal Groups

There are more than a dozen varietal groups in peppers, and new ones are still being created by breeders. Once again, the preponderance of varietal types falls under the

C. annuum species. *C. chinense* and *C. baccatum* have also been selected enough to distinguish several varietal groups in these species as well. Varietal groups are mostly distinguished by fruit size, shape and pungency, but colour may also be the key trait for classification as in the yellow wax types. Some colloquialisms used to describe varieties in the U.S. or elsewhere may actually confound matters. An example would be Chilli, which is derived from the Mexican word for all peppers-Chile. In the U.S. and elsewhere, it is applied only to long, pungent types. Most South Americans refer to all peppers (presumably mostly *C. chinense* and *C. baccatum*) with the word ají. Despite this, the most widely cultivated *C. chinense* types are called Habaneros or Scotch Bonnets. To avoid complete confusion, we will confine the following variety descriptions to the common commercial types of the U.S. and Mexico. For a more extensive discussion of pepper varieties, please refer to: *Peppers- the Domesticated Capsicums* by Andrews (1984) and *Peppers: Vegetable and Spice Capsicums* by Bosland and Votava (2000). Figure 1 demonstrates many of the popular pepper types cultivated in the U.S.

Fig. 1. Some common pepper varietal types: 1. Jalapeño, 2. Long Hot, 3. Orange Habanero, 4. Yellow Habanero, 5. Red Habanero, 6. Scotch Bonnet, 7. Long Hot Wax, 8. Antohi, 9. Hungarian Hot Wax, 10. Santa Fe Grande, 11. Pepproncini, 12. Cascabella, 13. Cherry, 14. Tabasco, 15. Pasilla, 16. Cayenne, 17. Serrano, 18. Mulato, 19. Poblano, 20. Pimento, 21. Hungarian Sweet, 22. White bell, 23. Purple bell, 24. Cubanelle, 25. Blocky bell, 26. Elongated bell, 27. Lamuyo, 28. Paprika.

3.1 Bell

These include large, often blocky and thick fleshed (5-10 mm) fruit which tend to be completely non-pungent. The fruit range from 100-120 mm in width and 100-200 mm in length. Most possess 3-4 lobes which are rounded or blunt at the base, leading to a blocky appearance. In most of the world, they are the leading sweet pepper for fresh consumption and dominate the greenhouse industry. Fruit are frequently harvested at the full-sized green stage for the fresh market, but their popularity as fully mature, coloured stage has increased dramatically with the advent of widespread greenhouse production. The mature colour may be red, yellow, orange or even white, depending on the allelic states of various genes which code for enzymes in the carotenoid biosynthetic pathway (Hernandez and Smith, 1985). There are too many varieties to mention here, but early development of open-pollinated types resulted in cultivars such as Keystone Resistant and California Wonder, which have been utilized in so many breeding programs for fruit quality, that they are likely the progenitors of many modern hybrids and disease resistant cultivars. Modern selections of California Wonder and newer open-pollinated cultivars such as Jupiter are still important in many U.S. production regions. However, much of the commercial production, particularly for the premium fresh-market trade, has switched to hybrid varieties, which have improved fruit quality, earliness, yield, and plant vigour. The greenhouse industry has led the way in hybrid bell utilization, resulting in the development of a highly lucrative seed trade, with prices often exceeding those of open-pollinated types by a factor of 10 or more.

3.2 Pimento

This group shares many of the characteristics of bells- thick-fleshed, non-pungent and large fruit. These types tend to produce heart-shaped or conical fruit, which are 50-100 mm in diameter at the top and taper to a point. Flesh thickness varies from 4-10 mm, depending on the intended market. Many of the European sweet peppers fall into this group, and may be used at the immature stage as a fresh vegetable in salads or stews. Mature fruits of either red or yellow colours are very frequently utilized for processed products such as pickles, roasted peppers or to stuff olives. The higher sugar content of the mature fruits imparts the desired flavour to these processed products.

3.3 Anaheim or Green Chile

These peppers are somewhat unique to the United States, though similar types called Chilacas and Guajillos are cultivated in Mexico. The early green chile industry can be traced back to farms established by Mexican Americans in New Mexico and other southwest states, during the 19th century. Early varieties including Sandía and Rio Grande, were highly variable for yield and fruit quality. Most produced moderately pungent, long (120-160 mm), smooth, thin-fleshed (2-4 mm) fruit, utilized for dried chilli or as a fresh cooking ingredient. Modern varieties, such as Anaheim and New Mexico 6-4, have more uniform, larger fruit, borne on tall (75 cm), vigorous plants.

Fruit mature from medium green to bright red. The red colour is a result of the ratio of Capsanthin to other carotenoids, and is a key quality attribute, measured by the American Spice Trade Association (ASTA). The fruit are utilized for both red and green processed products, such as chilli powder, roasted green chiles, and assorted cooking sauces for southwest cuisines. Fresh fruit are marketed for use as chiles rellenos, a popular stuffed pepper dish. Most are suitable as dual use varieties, though efforts to breed cultivars for specific products, such as canned green slices, have been successful. The thick-fleshed variety- Arizona 20, has become popular with the canning industry, while thinner-fleshed, more pungent types, are popular with the red chile industry. The Sonora variety is a popular dual use type. The Guajillo of central Mexico is a small green chile, which produces a powder considered to be the highest quality in that country. The Pasilla pepper, also of Mexico, matures to a dark chocolate colour, and when dried produces a richly flavoured powder, which is a key ingredient in some regional cuisines.

3.4 Ancho or Poblano

These large, dark green peppers are the most popular type in Mexico for the domestic market. In the fresh form, called Poblanos, they are utilized for the popular chile relleno dish. The dried form, Ancho, is commonly used to flavour many Mexican dishes, including the combination with chocolate called Mole. The mildly pungent fruit tend to be 2-3 lobed, conical or flattened in shape, with 3-6 mm thick flesh, and 120-160 mm long. Many have a characteristic sunken shoulder where the stem calyx attaches to the fruit. The ancho types tend to mature to deep red, while the mulato types mature to a chocolate brown colour. Limited production also occurs in the U.S., and large numbers of the fruit are imported to that country for the purpose of making chiles rellenos. The majority of production in Mexico relies on landraces, but recently introduced hybrids such as Tiburon and Ancho Villa, have garnered a significant share of the seed market.

3.5 Paprika

This refers to a medium length, light green pepper, grown exclusively for the production of high quality chile powder. The most common type is the Hungarian, which has smooth fruit, with thin flesh (1-3 mm), and a length of 80-120 mm. In Spain, much variability exists in fruit size and shape, with most being flattened, conical fruit with little or no pungency. Types grown in the U.S., such as B58 and Garnet, tend to be longer and likely have some green chile in their pedigrees. These are probably the most industrialized of all peppers, with mechanization involved in all aspects of production. All have been selected for high ASTA values and corresponding bright red coloured powder. In Spain, these peppers are often dried by smoking, imparting a unique flavour, which is popular for certain cuisines.

3.6 Cayenne

These medium-green peppers are generally long (200-250 mm), and narrow (10-20 mm) with moderate to high pungency levels. Plants are tall and spreading, with multiple branches. Unlike green chiles, they tend to be quite rough, wrinkled or even twisted, with thinner flesh (1-2 mm). Most mature to dark red, though some may mature to orange or yellow. The uniquely sweet and spicy fruit are utilized to produce fermented mash for Louisiana style, hot pepper sauces, which are extremely popular in the U.S. They are also dried to produce hot chile powder or pepper flakes, which are a popular condiment. The green fruit are sold as "long hots" or frying peppers in some regions, such as Italy and the U.S. Like the paprika, production of this pepper is highly industrialized for processing purposes.

3.7 Tabasco

This pepper deserves mention due to the tremendous influence it has had on the consumption of hot pepper products worldwide. It is the only cultivar of *C. frutescens* cultivated commercially on a significant scale. The Tabasco brand of hot pepper sauce is the world's most popular, utilizing the small (20-30 mm), bullet-shaped pepper, which matures from yellow-green, to bright red. The fruit is produced on a tall (800-1000 mm), vigorous plant, which grows as a perennial in tropical locations, allowing multiple harvests. The immature fruit are also used to make pickles and hot vinegars.

3.8 Jalapeño

This group includes the most commercially important hot pepper cultivars in the United States and Mexico. The fruit tend to be bullet-shaped, either blunt-nosed or slightly pointed. Most have 2-3 locules, though occasional fruit may have four. Length and width are quite variable among the many cultivars, ranging from 50-100 mm long and 20-40 mm wide. Flesh thickness is generally between 10-20 mm. Immature colour ranges from light to dark green and some cultivars have numerous cuticular cracks in the exocarp. Mature colour is mostly dark red, but can also be yellow or orange. Plants are generally small to medium in height (300-500 mm), with 1-2 main stems that produce multiple lateral branches. Green fruit are the predominant fresh hot peppers in North American markets, and are also processed extensively into pickles, salsas, sauces and powders. Red fruit are utilized for powders, sauces and the popular smoked preserved peppers called chipotle. Many varieties have been bred for different markets and production regions, with non-pungent, mild and hot fruits. Hybrid cultivars now dominate the industry, fostering a lucrative seed trade and large commercial breeding programs.

3.9 Hot Chiles

This group includes a wide range of varieties with similarly shaped, thin, tapering fruit with high pungency levels. Most are produced by tall (80-100 mm) plants, with

multiple branches and often pubescent foliage. The smooth fruit range between 50-120 mm in length and 8-12 mm in width. The Korean and Indian hot red chilli types tend to be longer than the Thai varieties or the Mexican chile de arbol types. The Mexican Serrano pepper, generally has thicker flesh (3-5 mm) and is mostly utilized as a fresh green chile for salsas or pickles. The Asian types are utilized both as red fruit for processing into sauces and powders, or as green fruit for cooking and salads. Hybrid cultivars of many hot chilli types have recently begun to dominate the markets in many countries, reducing the variability associated with some traditional varieties.

3.10 Yellow Wax

These peppers take their name from their light yellow, waxy appearance at the immature stage. They may be conical or jalapeño-shaped, such as the Santa Fe Grande or cascabella types. Or they may be long (100-200 mm) and cylindrical, such as the banana and Hungarian types. Flesh thickness is variable (5-8 mm), but usually greater in the short types. They may be sweet, such as banana, or cubanelle types, or extremely pungent, such as cascabella. Most mature to bright red, but some turn yellowish-orange. They are primarily produced for processing into pickles in the U.S. and Mediterranean countries. However, there is a fresh market demand for use in salads and various cuisines, especially in parts of Eastern Europe and the Middle East. Some wax types are particularly high in anti-oxidant compounds, such as flavonoids and ascorbic acid.

3.11 Habanero

In this group we will include both Habanero and Scotch Bonnet, the commercially important types of *C. chinense*. All grow on a large (800-1200 mm), bushy plant, which is a perennial in the tropics, and is harvested multiple times. Habaneros typically have a lantern or blocky shape, 30-50 mm in length, and 20-30 mm in width. Scotch Bonnets are usually like flattened tops, wider (30-50 mm) than their length (25-40 mm). Fruit range from light to dark green immature, and may turn deep orange, yellow, red or chocolate coloured. Most are consumed at the coloured stage, and possess a unique, fruity flavour, sometimes accompanied by a musky aroma. There are many different shapes and sizes intermediate between these two types throughout tropical America. Most are extremely pungent, though some are mild or even sweet. Uses include pickles, sauces, powders and as a fresh ingredient for salads or cooked dishes. The fruit is also used to extract capsaicin for industrial purposes.

4 Genetic Resources

There are 25 species in the genus *Capsicum*, spread throughout the new world tropics, from south Texas to Argentina (IBPGR, 1983). The primitive types in the cultivated and wild species tend to have small, seedy fruit, with thin flesh. This

probably aids in dissemination by birds and other creatures which feed on the fruit. Lippert et al.(1966) listed several descriptors for distinguishing between the species based on flower morphology. Extensive wild and improved germplasm of the five cultivated species- *C. annuum, C. chinense, C. baccatum, C. frutescens,* and *C. pubescens* exists throughout tropical America. Mexico is the primary centre of diversity for *C. annuum*, with secondary centres in Central America and northern South America (Andrews, 1984). Other secondary centres of diversity for *C. annuum* include India, the Mediterranean region and Southeast Asia. Landraces of this species have been developed by farmers in both lowland and highland regions. The greatest genetic diversity among the other four cultivated species is found in South America . *C. pubescens* is restricted to high elevation regions in Bolivia and Peru, preferring cool conditions (Bosland and Votava, 2000). The other three species are at home in the lowland tropics of the Amazon basin, where many wild and cultivated types may be found. The lowlands of the Caribbean islands are an important secondary centre of diversity and domestication for *C. chinense*.

Both private and public interests have created extensive germplasm collections of the cultivated species in these regions over the past 100 years. Perhaps the largest single collection of *Capsicum* species in the world is the USDA-ARS-GRIN. The repository at Griffin, Georgia, holds over 5000 accessions of the cultivated and wild pepper, species, with nearly 4000 of *C. annuum*. This includes both wild germplasm collected in the countries of origin, and improved cultivars developed by plant breeders. The INRA of France also maintains an extensive collection of *Capsicum* germplasm. The European Cooperative Program for Crop Genetic Resources has established a system of core facilities to consolidate germplasm collections from around Europe and the Russian Federation (Maggioni, 2004). The *Capsicum* collection is located at Aegean Agriculture Research Institute, Izmir, Turkey. The University of Torino, Italy, holds a duplicate of the *Capsicum* Genetic Cooperative germplasm collection.

5 Major Breeding Achievements

The traits of interest to pepper breeders are the same as those for most important vegetable crops. These include fruit quality, disease resistance, earliness and vigour, yield, stress tolerance, and plant morphology. The progress during the latter half of the 20th century in developing commercially important cultivars of pepper has been remarkable. This was driven by a combination of increased consumer demand for high quality products, and expansion of the commercial vegetable seed industry on a global scale. The development and distribution of hybrid pepper seed has led to an explosion of new cultivars during the last 20 years. However, the importance of open-pollinated pepper variety development cannot be diminished, as such types served as the genetic base for most modern cultivars. In many countries, open-pollinated cultivars still comprise a major portion of the commercial production. Many of the achievements mentioned here will indeed pertain to open-pollinated cultivar development. There are far too many specific traits of interest to pepper breeders or growers around the world to mention in one chapter. Therefore, this text

will address some truly significant achievements, which have application in many commercial pepper production regions.

5.1 Fruit Quality

The most obvious trait of interest to breeders and growers is uniformity. Because they are a self-pollinating crop, this has been accomplished by inbreeding peppers, while selecting for important shape, flavour, appearance and yield traits by breeders throughout the world. Important early cultivars include Hungarian hot wax, Chinese Giant Bell, California Wonder Bell, and Long Red Cayenne. These were selected for uniform fruit quality and adaptation to various important production regions. The popularity of sweet bell types led to the development of cultivars with many different fruit attributes. These included- blocky 4-lobed, thick flesh, red, yellow, orange and brown mature colour, green, white and purple immature colour, elongated or Lamuyo types and even miniature, ornamental cultivars. Important cultivars include Yolo Wonder, VR-4, Jupiter, Grande Rio 66 and hundreds of hybrid cultivars. Fruit quality traits important for hot red peppers such as cayenne or Asian types, include high pungency, high ASTA, pleasant aroma, thin flesh and good dry matter content. Cultivars include Long Red Cayenne, Durkee Cayenne, Santaka, Thai Hot, and many new hybrids. These also apply to green chile and paprika types, except pungency. Important cultivars include Anaheim, New Mexico 6, Conquistador, Sonora, Garnet, and Hungarian. Hot peppers for the fresh market must have thick, firm flesh and moderate pungency levels. High yield, earliness and large fruit are also important traits bred into modern varieties. The first jalapeño cultivars to include many of these traits were Early Jalapeño, Jalapeño M, TAM Mild Jalapeño, and Vera Cruz. Modern hybrid cultivars are even more uniform for fruit size, shape, plant vigour and yield. These include Grande, Mitla, Tormenta and many others. Increased pungency and crackless skin are traits, which have become crucial for fresh market cultivars today. Only recently have serrano peppers received much attention for cultivar development. The same traits important in jalapeño should be found in serrano. Cultivars include Hidalgo, Huasteco, Tampico, and Tuxtlas.

Another facet of fruit quality only now receiving serious consideration is nutritional value. Peppers are potentially an excellent source of anti-oxidant compounds and phytochemicals which affect human health. Varieties with uniformly high levels of ascorbic acid, flavonoids and carotenoids are now being developed (Crosby et al., 2005b, 2005c).

5.2 Disease Resistance

5.2.1 Viruses

The greatest advances in pepper disease resistance breeding have been related to viruses. Many types of viruses infect pepper plants, with some very thoroughly investigated and others yet to be identified. Three virus families have been implicated in mosaic diseases of pepper throughout the world. These are tobamoviruses, potyviruses, and cucumoviruses. Members of the begamovirus and

tospovirus family have begun to receive more attention in the last 20 years for their severe impact in certain pepper production regions. The first virus to receive much attention from the standpoint of developing host plant resistance was Tobacco Mosaic Virus. This virus is transmitted mechanically and by seed contamination. A single, dominant resistance gene was identified in the Tabasco pepper (*C. frutescens*) by Holmes (1937), and named *L*, for local lesion type resistance. He introgressed this gene into bell pepper. Many modern cultivars now contain the *L* gene, though it has been renamed *L2* (Boukema, 1980). Strains of TMV which were able to overcome this gene were identified in several U.S. locations (McKinney, 1952; Greenleaf, 1986). These have since been named Pepper Mild Mottle Virus. A dominant resistance gene to PMMV was identified in *C. chinense*, and named *L3* since it was allelic to *L2* (Boukema, 1980). Resistance breaking strains have since been identified and there are no modern cultivars resistant to all strains. Some *C. chinense* and *C. baccatum* accessions demonstrate apparent immunity or mild symptoms (Greenleaf, 1986; Author, unpublished data).

The potyviruses have also been implicated in numerous pepper diseases, with dramatic impact on yield and quality in production regions worldwide. Symptoms are usually leaf chlorosis, with leaf and fruit mottling and deformation in severe cases. Greenleaf identified a single recessive gene for resistance to Tobacco Etch Virus in *C. annuum*, SC 46252, and *C. chinense,* PI 152225, and named them et^a and et^c (1956). Cook (1960) identified resistance to Potato Virus Y (PVY) and some strains of Tobacco Etch Virus (TEV) in *C. annuum,* PI 264281, which turned out to be allelic to et^a. A third recessive gene which conditioned resistance to Pepper Mottle Virus (PepMoV) was identified by Zitter and Cook(1973), and named et^{av}. These genes all provided resistance to PVY as well. A single dominant gene from Criollo de Morelos 334 was found to confer resistance to both PVY and PepMoV (Dogimont et al., 1996). Kyle and Palloix (1997) proposed a standardized system of nomenclature for potyvirus resistance genes, calling them pvr1, pvr2, etc. The first three recessive genes discovered- et^c, et^a and et^{av}, have been introgressed into many different pepper types over the past 40 years. These included the VR series of Cook, Tabasco Greenleaf, and numerous hybrid cultivars. Villalon pyramided et^a, et^{av}, and some uncharacterized resistance genes from wild serrano types into a number of hot pepper cultivars, including jalapeños, serranos, hot wax and green chiles (Villalon, 1986; Villalon et al., 1994). Ariyaratne et al.(1996) screened many of these cultivars and resistance gene sources against 36 strains of TEV from around the world. They identified resistance breaking strains, but also some accessions with resistance to many strains. It appears that the combination of et^c, et^a and et^{av} might provide resistance to all of the diverse strains tested. Such pyramided resistance breeding seems to be a practical approach to assemble horizontal resistance and is currently underway in the author's breeding program for jalapeños.

There are many strains of Cucumber Mosaic Virus, and some cause severe symptoms when they infect peppers. In most of Asia, CMV is the most destructive virus in peppers. Symptoms include mosaic, narrowing and deformation of leaves, chlorotic spots on leaves and fruit. It is transmitted by a number of aphid species and also by mechanical means. Resistance has been identified in Perennial (*C. annuum*) and some other Asian germplasm accessions (Singh and Thakur, 1979; Monma and

Sakata, 1997). However, in Perennial, the resistance appears to be strain specific and possibly polygenic recessive (Green and Kim, 1991; Pochard and Daubeze, 1989). Another source of resistance was identified by Grube et al. (2000), in *C. frutescens*. They concluded that at least two recessive genes were involved, but in certain F_2 families, incomplete penetrance hindered resistance expression. Resistance identified in some *C. baccatum* accessions has been utilized in France and Hungary to develop some tolerant cultivars in those countries. Overall, the poor heritability of CMV resistance has hindered variety development for this disease. However, a few commercial cultivars have been released with some level of resistance, including-Emerald and Revolution.

The Tospoviruses comprise another economically important group of viruses which dramatically impact many vegetable species. Tomato Spotted Wilt Virus has become a serious threat to pepper cultivation in the U.S., Mexico, and Mediterranean regions. This virus is transmitted by thrips, flea beetles and a few other insects. Symptoms include mosaic, leaf and fruit ringspots and deformation. This virus has many hosts, including tomatoes, peanuts and other crop plants often grown in the vicinity of pepper fields. Resistance was identified in *C. chinense* and determined to be local lesion response, conditioned by a single dominant gene (Black et al., 1991; Moury et al., 1997). The *Tsw* gene has been introgressed into *C. annuum* types, including commercial bells such as Stiletto. However, it is strain specific and has been overcome in Italy and other locations (Moury et al., 1997).

The Begamoviruses are a very large family of DNA viruses, which infect many important vegetable crops. Most are vectored by whiteflies in the tropical regions of the world, though some may be transmitted by leaf hoppers. In the western U.S., beet curly top is a significant problem in peppers, particularly direct seeded crops. If infection occurs early, stunted, chlorotic plants will produce no marketable yield. Potential resistance was reported in several USDA Plant Introductions by Ungs et al (1977), but was related to reduced numbers of leaf hoppers. Bosland (2000) reported several accessions of *C. annuum*, and three other species which demonstrated low incidence of curly top symptoms in a New Mexico field trial. Because no immunity response was found, the mechanism may likely be non-preference or anti-biosis of the leaf hopper vector. Many whitefly transmitted viruses have been reported from pepper plants in Mexico, India and other tropical regions. These include Huasteco virus, Serrano Golden Mosaic and Chilli Leaf Curl (Black et al., 1991). No resistant cultivars have been bred, though a few reports of resistant germplasm from Mexico have been published (Godinez-Hernandez et al., 2001).

5.2.2 Bacterial Leaf Spot

This widespread disease of peppers is caused by *Xanthomonas campestris* pv. *vesicatoria*. It is most severe in humid climates and may develop in explosive fashion following rainy periods. The southeastern United States, Southeast Asia and Mexico are hotspots for this disease. It is not a major threat in arid regions, such as New Mexico or the Middle East, unless inoculum is introduced on seed, and favourable environmental conditions are created by poor cultural practices. The inoculum may remain in leaf litter and plant material for months, particularly in

humid climates. It also infects the seed coat and has been spread by humans around the globe on untreated seed. Resistance was first identified by Cook and Stall (1963) as a hypersensitive response in PI 163192, which was determined to be conditioned by a single dominant gene, *Bs1*. This resistance only worked against race 2 of the pathogen in Florida. A second, dominant gene from PI 260435, which conditioned hypersensitive resistance to race 1, was later identified by Cook and Guevara (1984), and called *Bs2*. Both of these genes were introgressed into numerous cultivars, mostly bell and pimento types (Greenleaf, 1986; Jones et al., 1998). A third gene, *Bs3*, was identified in PI 271322. Hibberd et al (1987) confirmed the distinct nature of these three genes, and demonstrated that *Bs2* resisted both strains. Later, resistance breaking strains were identified, and there are at least 11 races, responding differently to the three genes. Race 6 was the first to overcome all three resistance genes, though an accession of *C. pubescens* was identified, which carried a new dominant gene (*Bs4*) for resistance to that race (Sahin and Miller, 1998). Race 10 apparently overcomes all four dominant genes (Ritchie et al., 1998). Recently, recessive, non-hypersensitive resistance genes, have been identified and utilized to create new resistant cultivars. This resistance is associated with low levels of bacterial multiplication on the leaves. Jones et al. (2002) named two of these *bs5* and *bs6,* and demonstrated that they act in concert to reduce BLS on the leaves. The non-hypersensitive resistance may be less likely to be overcome by mutations in the pathogen if avirulence genes are not involved. Many accessions of *C. chinense* and *C. baccatum* have very low incidence of BLS under heavy disease pressure in south Texas, suggesting some mechanism of horizontal resistance (Author-unpublished data). Resistant cultivars carrying *Bs2* are widely deployed by growers in the western hemisphere. These include mostly bells- Wizard X3R, Aristotle X3R, Crusader and Summer Sweet series. Recently, some jalapeño and wax cultivars carrying *Bs2,* have been commercialized. These include- Ixtapa, Tormenta, El Rey and Hot Spot.

5.2.3 Phytophthora Root and Stem Rot

The soilborne pathogen, *Phytophthora capsici*, is widespread in many pepper production regions, causing rapid plant decline and serious yield reductions. Many soils in Mexico and elsewhere have been abandoned to pepper cultivation, due to high inoculum levels. The pathogen thrives in wet soils, requiring free water for zoospores to move about and contact root tissue. In many fields, poor cultural practices, such as over irrigating, failure to control weeds, or impeding proper drainage, lead to severe Phytophthora outbreaks. Due to the fact that many pepper production regions have infested soils, host plant resistance would seem to be the best solution. Resistance has been identified in several small, hot chile types, but introgressing the genes into bells or other commercial types has proven difficult. Resistance in *C. annuum*, PI 201234, was identified by Kimble and Grogan (1960), and determined to be conditioned by one or two dominant genes (Smith et al., 1967). This resistance has been incorporated into some French and U.S. cultivars, but has not held up in all locations, likely due to genetic variability in the pathogen (Lefebvre and Palloix, 1996). A higher degree of resistance was identified in Criollo de Morelos 334, but it is linked to poor fruit quality traits. This resistance is likely

polygenic, as even extremely large F_2 families may not contain individuals as resistant as CM 334 (Berke- personal communication). A third source of resistance was found in a small orange chile, Fidel, and introgressed into jalapeño lines. This resistance was determined to be conditioned by two recessive genes (Gonzalez, 2002; Author, unpublished data). Tolerant commercial cultivars include- Revolution and Paladin.

5.2.4 Powdery Mildew

This disease, incited by *Leveillula taurica*, has become a widespread problem in field cultivated peppers around the world. Symptoms include small tan lesions, followed by powdery hyphal growth on the leaf surface. It seems to prefer arid regions with artificially elevated humidity due to irrigation or greenhouse culture. It is inhibited by excess foliage moisture and therefore not as common in rainy regions. In severe infestations, leaf defoliation leads to sun-scalded fruit and crop loss. In some regions, the pathogen has developed resistance to fungicides, making host plant resistance a desirable alternative. The disease is most severe in bell, wax and jalapeño peppers. Many *C. chinense* and *C. baccatum* types seem to be either immune or poor hosts for the pathogen. Resistance has been described in some *C. annuum* sources (Desphande et al., 1985). The line HV12 from INRA, derived resistance from an African pepper, and has been utilized in breeding programs in France and Israel (Daubeze et al., 1995; Shifriss et al., 1992). This polygenic resistance does not appear to hold up well to the pathogen races in Texas or Israel (Coffey and Author- personal communication). Other sources of resistance have been identified by screening with a highly virulent California isolate (Coffey 2005). Resistance in *C. chinense* has been transferred into a jalapeño background by the author, but extremely low heritability suggests multiple recessive genes or incomplete penetrance.

5.2.5 Root Knot Nematodes

Root knot nematodes (*Meloidogyne* spp.) are a serious pest of peppers in some soils around the world. Stunted plants in infested soils will suffer considerable yield reduction, if not total crop failure. Resistance to the southern root knot nematode was identified by Hare (1957) in Santaka and a Mexican accession 405. He determined that both carried a single dominant gene and named it *N*. He introgressed it into a pimento background and released Mississippi Nemaheart, pimento pepper (Hare, 1966). Later, Fery and Dukes identified a second, recessive resistance gene, which was present in Carolina Hot, along with the N gene. Fery et al. (1986) then developed Carolina Cayenne, utilizing these resistance genes. This was followed by Carolina Wonder and Charleston Belle, both carrying the N gene (Fery et al., 1998). The resistance conditioned by the *N* gene was later found to be less effective at high temperatures (Thies and Fery, 2002). More recently, Fery and Thies (1998) characterized the resistance to *M. incognita* in *C. chinense* germplasm, and found it to be conditioned by a single dominant gene, allelic to the *N* gene from *C. annuum*. This resistance gene was introgressed into a Habanero background, and released as a new cultivar, Tigerpaw (Fery and Thies, 2006). Because the N gene does not

condition resistance to the northern RKN (*M. hapla*), an effort to screen *Capsicum* spp. germplasm was undertaken to identify other sources of resistance (Thies and Fery, 2002). Out of 440 accessions, none were resistant, though a few had lower levels of nematodes than susceptible checks.

5.2.6 Other Diseases

There are many other serious diseases of pepper that cause crop losses in different regions of the world. However, development of resistant cultivars has not been as productive as for the major diseases mentioned above. Resistance to bacterial wilt (*Pseudomonas solanacearum*) is an important issue in Southeast Asia and some level of resistance has been identified in germplasm from Japan, Korea, and India. Resistance was verified in the cultivars- Mie Midori, Tachi-Tatsubusa, Weonkyo 306 and Baramashi by Matsunaga and Monma (1999). Rhizoctonia root rot causes disease on peppers in some regions of the U.S. and other countries, mostly on heavy soils. Resistance was identified in several capsicum species by Muhyi and Bosland (1995), but no resistant commercial cultivars have been developed.

5.3 Male Sterility

Another trait of interest to vegetable breeders is male sterility. In the absence of viable pollen, hybrid seed may be generated without contamination from selfing. This allows for exploitation of heterosis in F1 hybrid cultivars, and also permits breeders and seed producers to protect their unique inbred lines from unlicensed reproduction. In pepper, both genic male sterility and cytoplasmic male sterility have been described (Shifriss, 1997). Two distinct recessive genes for male sterility were described by Shifriss and Frankel (1969), and Shifriss and Rylsky (1972). Because of the expected Mendelian inheritance of 25% for homozygous recessive genotypes upon selfing, the preferred method was to cross a homozygous recessive sterile plant with pollen from a heterozygous sibling with uniformity for other important traits. This would generate 50% sterile plants for seed production after roguing. This system has been utilized to generate hybrids by commercial seed companies, for both bell and hot pepper types (Terry Berke-personal communication). A more effective system in other crops, such as onion, has been cytoplasmic male sterility. This requires the interaction of a sterile cytoplasm and a recessive sterility gene. Seed of the sterile parent lines can be created by crossing with a sibling maintainer line which is uniform for the other important traits, homozygous recessive for the male sterility gene, but containing normal cytoplasm. Hybrid seed is then produced by using a restorer line as the pollen parent. This may be any line which is homozygous for the fertility allele, regardless of cytoplasm type. Unfortunately in peppers, the CMS system has not been very useful, due to its instability. Fertility seems to be restored at cooler temperatures. Also, the genetic background of the CMS line and the maintainer appear to dramatically influence the expression of the sterility trait (Shifriss and Guri, 1979). Recently, a gene affecting stability of CMS, *St*, has been described by Lee et al (2005). In addition, molecular markers have been developed which discriminate between sterile and fertile cytoplasms (Kim and Kim, 2005).

However, due to the difficulty in developing stable CMS, commercial utilization of the system in pepper remains rare.

6 Current Goals of Breeding

There are many objectives in a typical pepper breeding program, some of which represent traditionally important traits and others which have arisen due to emerging diseases, horticultural issues or market trends. Current goals for fresh market pepper breeding are somewhat different than those for processing or spice markets. The trend in fresh peppers has been to larger sized fruit, with less external blemishes and more uniform colour and shape. This means 10 cm x 10 cm, blocky, 4-lobed bell peppers, with medium-green, thick flesh (8-10 mm) and no anthocyanin purpling in the epidermis. This applies to green and mature, coloured bells in the U.S. However, coloured bells of the Lamuyo, or elongated type are the target of many European breeding programs. These are much longer (10 x 18 cm) than wide and may be 2-4 lobed, and even slightly pointed, rather than rounded at the base. Jalapenos should be at least 7-8 cm in length, with rounded shoulders, and smooth, dark green epidermis. Asian green chiles should be thinner fleshed than serranos or jalapeños, and wrinkled epidermis is acceptable. Breeding for uniform pungency is crucial to the fresh market, where consumers expect Poblanos to be mild, jalapeños to be moderately pungent and Habaneros to be extremely pungent. Milder versions of hot peppers have been bred and may appeal to markets not accustomed to high pungency. The breeding program at the Texas Agricultural Experiment Station has led the way in developing mild cultivars of jalapeño, serrano and Habanero (Villalon, 1983; Crosby and Villalon, 2002; Crosby et al., 2005a).

Another aspect of fruit quality receiving greater attention from breeders is flavour, which is independent of pungency. The great number of aromatic compounds, sugars and acids which affect pepper flavours makes breeding for enhanced quality somewhat subjective. No standards exist for what the ideal flavour profiles should be for jalapeño, pimento or other pepper types. Therefore, large breeding populations and multi-location trials are crucial to assess flavour attributes of modern cultivars. Some breeding programs are beginning to focus on genetic control of phytochemicals other than capsaicin. Many of these have potential human health benefits, including- flavonoids, phenolic compounds, ascorbic acid and carotenoids (Howard et al., 2000). Investigations at Texas A&M University have demonstrated extensive genetic variation for levels of these compounds in different pepper germplasm. New cultivars with genetically enhanced levels of carotenoids and flavonoids are under development (Crosby et al., 2005b).

Solutions to many of the diseases mentioned above have been found through the use of resistance genes. However, new strains or races of viruses, bacteria and fungi are constantly developing. Therefore, identification and exploitation of new resistance genes remains one of the most important goals of pepper breeding programs in the 21st century. This includes screening germplasm to identify resistance to newly emerged diseases as well. Much less focus has been given to identifying genetic mechanisms of insect resistance in peppers. The tremendous

destructive capacity of pepper weevils, thrips, aphids, whiteflies, lepidopteran species and mites should not be overlooked. The production of peppers often requires frequent applications of pesticides, leading to very high expenses for the growers and potential for environmental pollution. Resistances to some arthropod pests have been observed, but more characterization of the genes involved and exploitation by breeders needs to be done (Kashiwagi et al., 2005; Maris et al., 2003). Yield and stress tolerance are other goals of many pepper breeding programs. Many cultivars bred in moderate climates may not perform well in tropical or desert production regions. Both cold and heat tolerance are traits which have been addressed by some breeders. High temperature fruit set has been bred into some cultivars developed at the TAES. This results in improved yields over other cultivars in hot climates. Equally important may be seedling cold tolerance for early planting regions or northern latitudes. Breeding for drought-tolerance is also important, considering the increased urban demands on water supplies in many arid regions where pepper cultivation occurs. Germplasm from arid regions of Mexico, such as pequin, Serrano and Guajillo, may hold promise for identifying drought tolerance related genes. Traits which may be associated with drought stress tolerance in peppers include foliage size, colour and pubescence, photosynthesis efficiency and root system size (Author-unpublished data).

Processing pepper cultivars are not subject to the strict requirements for appearance, but need to produce high yields, with uniform pungency. Thick-flesh, reduced seed cavity size, and easy de-stemming are desirable traits for hot green cultivars, such as jalapeño. Ease of peeling the epidermis is important for green chile cultivars, or pimentos to be canned. Cultivars for chile powder production should have high dry matter percentages, high ASTA if red-fleshed, and relatively uniform capsaicin concentrations. Variability still exists for these traits in many open-pollinated cultivars, but blending can compensate. All of these traits need to be integrated into hybrid cultivars by most modern pepper breeding programs. This facilitates protection of proprietary germplasm and allows exploitation of hybrid vigour for yield, earliness and fruit quality. However, it makes disease resistance breeding more difficult, as many genes may be involved. In addition, unlike in tomato, many important resistance genes in pepper are recessive, requiring both elite inbreds to be homozygous at the loci of interest. Integration of molecular techniques, such as marker-assisted selection and genetic transformation are also becoming key issues in modern breeding programs.

7 Breeding Methods and Techniques

The cultivated species of pepper have perfect flowers and are considered a self-pollinating crop. No inbreeding depression has been reported among open-pollinated cultivars, but the author has observed a gradual reduction in plant height following repeated selfing in a single seed descent method. Cross pollination may be high in some peppers, depending on bee activity and the morphology of the style and stigma. If the stigma is exserted beyond the anthers, cross-pollination may be more common, as peppers tend to be protogynous. Pickersgill reported cross-pollination rates

ranging from 2% to 90% in peppers (1997). Several techniques may be used to prevent cross-pollination. The most common is planting uniform lines in isolation plots. These may be isolated by distance or by insect proof nets or cages. Another technique is hand emasculation of individual flowers, followed by manual self-pollination with viable pollen from the same plant, then covering the flowers or taping the petals shut. The same technique is used to manually cross pollinate when creating hybrids or backcrosses, with caution taken to prevent contamination by alcohol dips or wipes. Pollen may be kept in small plastic micro-centrifuge tubes, glass slides or other appropriate containers if many crosses are to be made. If only a few crosses are to be made, individual flowers of the donor parent may be used as miniature brushes to transfer the pollen to the emasculated seed parent stigmas. The best time to hand pollinate is in the morning hours, to avoid heat associated stress and reduced stigma receptivity. In a greenhouse, field or cage, insecticides should be applied to control ants, beetles, bees and other insects which could potentially contaminate either the stigma or the pollen parent anthers. The control of aphids and whiteflies should also be a priority to avoid infection of seed parent plants with viruses. Sanitation of seeds, pots and all greenhouse facilities is also crucial to avoid outbreaks of BLS and PMMV. Bleach and TSP are good choices to sanitize forceps, gloves, and other items, while alcohol may be safer for hands.

There are many breeding schemes which may be applied to peppers. These include mass selection, pedigree, single seed descent, recurrent selection, backcross and mutation induction.

7.1 Mass Selection

This technique is usually chosen to improve populations for multiple traits at the same time, without concern about pedigrees. It is generally results in rapid improvement of the population for desired traits with limited expense (Fehr, 1987). It must be employed in an environment where the traits of interest are expressed. Effectiveness may be enhanced by rouging off-type plants prior to pollination, particularly in peppers, which may outcross readily. The earliest pepper breeders employed this technique to develop land-races or open-pollinated cultivars. Traits with high heritabilities are much easier to fix in the resultant populations. The maintenance of high levels of genetic variability is one advantage of mass selection. This technique is commonly used in Mexico to select seed for Poblano, guajillo and other traditional pepper land-races.

7.2 Pedigree Method

This technique involves rapid inbreeding through single plant selections at each generation. Many modern pepper cultivars and inbreds for F_1 hybrid cultivars are developed by this method. F_2 selections are made based on the desired traits, with careful records kept of each selection. This process continues for several generations until the desired level of homozygosity is reached. In peppers, steps to avoid out-crossing, such as cages or manual pollinations, may be taken. Usually, at least 6-8 generations of inbreeding are required to develop uniform lines. As in mass

selection, this technique requires an environment where the traits of interest are expressed. Extensive experience with phenotypic evaluation is required and large F_2 populations will facilitate selection for traits with low heritability or those under polygenic control. This method is often utilized in conjunction with backcrossing to introgress important genes into advanced inbreds or open-pollinated cultivars. Examples of cultivars developed by the pedigree method include Yolo Wonder, Conquistador and TAM Mild Jalapeño.

7.3 Single Seed Descent

This method is used to rapidly inbreed populations without much selection for traits of interest (Fehr, 1987). In peppers, it is often carried out in a greenhouse, or winter nursery, in order to advance more generations per year. Selection for seed viability and seedling vigour may be possible, as may selection for virus resistance or other single gene resistances amenable to controlled inoculation. Villalon (1986) employed this technique to fix recessive potyvirus genes in inbred lines, prior to testing them in the field for other traits of interest. This technique is widely employed now to generate large numbers of inbred lines to be used in testcrosses for hybrid cultivar development.

7.4 Backcross

The backcross method is used to introgress key genes from germplasm or distinct inbreds into advanced breeding lines or open-pollinated cultivars. It involves a recurrent parent, with desirable horticultural attributes and disease resistance and a donor parent, with a gene or genes of interest. After selection for the gene of interest in the F_2 population, backcrossing begins. If the key gene is dominant, direct backcrossing may be conducted with selection at each generation, predominantly for the new trait. If the gene is recessive, selfing should be undertaken, followed by selection, prior to each backcross. The desired level of similarity to the recurrent parent may be reached in 6-8 generations. This technique has been widely employed to introgress disease resistance genes into many pepper cultivars and inbred lines. The dominant *L* genes for TMV, the recessive *pvr* TEV resistance genes, and the various BLS resistance genes are all examples (Holmes, 1937; Greenleaf, 1970). Cultivars include Yolo Wonder R, Tabasco Greenleaf and Mississippi Nemaheart.

7.5 Recurrent Selection

This method involves selection of superior individuals in a population to create a new, improved population for the next generation. This method works for quantitative traits and those with low heritability. It has the advantage of maintaining genetic variability for important traits which can be selected later. The selection of open-pollinated seed is often referred to as phenotypic recurrent selection, and is commonly employed with peppers. Other schemes, employing controlled self-pollination or sib crossing, will result in more rapid progress in fixing traits of interest. However, much more work is involved. Recurrent selection is crucial to a

pepper breeding program, which much simultaneously select for many important genes, particularly those related to yield and other quantitative traits. The improved populations may serve as the foundations for other breeding schemes, by identification of superior individuals to use as elite parents. The author employs recurrent selection to develop elite populations for stress tolerance, yield and fruit phytochemical content.

7.6 Mutation

Mutation breeding is usually aimed at modifying select qualitative traits in a single generation, while maintaining as many other characteristics of an improved line as possible. It generally involves nucleotide deletions or chromosomal rearrangements, which deactivate specific genes. These genes may condition undesirable phenotypes or their absence may lead to novel quality attributes. This technique has been used sparingly in peppers, mostly by means of chemical mutagenesis (Bosland and Votava, 2000). Radiation may also be used to create chromosomal breakage and prevent expression of key genes. The process is random, but may be heritable and result in rapid phenotypic changes in a single generation. Large populations are necessary due to the high frequency of undesirable genotypes generated.

8 Integration of New Biotechnologies in Pepper Breeding Programs

Advances in biotechnology have allowed breeders access to unique traits not available within the germplasm of various plant species. These include genes from other organisms such as bacteria, viruses and insects. In addition, new gene mapping and cloning technologies have expedited the process of gene discovery and isolation. Many genes from *Arabidopsis* and other plants may be useful to impart desired traits in peppers in the future.

8.1 Gene Mapping

The use of molecular markers, such as randomly amplified DNA (RAPD), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), microsatellites and simple sequence repeats (SSR), to tag single genes or quantitative trait loci, will expedite the selection process. Many such markers in pepper have already been associated with traits of interest, through genetic linkage analyses in segregating populations. Pierre et al (2000) developed a linkage map around the *Bs3* gene, identifying flanking AFLP markers at about 1.0 cM. They converted these into locus specific, PCR-based markers for selection purposes. Tai et al (1999a) utilized chromosome walking and tightly linked AFLP markers to clone the *Bs2* gene in a F_2 pepper family. They then transformed susceptible tobacco and tomato to demonstrate the resistance function of the gene. PCR-based markers are now available to screen pepper progeny for the presence of the *Bs2* gene, eliminating time and money involved in controlled inoculations. This process is much more

efficient than raising plants, and inoculating them with a known race of bacteria. Figure 2 demonstrates the highly reproducible SCAR marker devised by Tai et al (1999b), as reproduced in the author's lab. In all cases, resistance should ultimately be confirmed by inoculation with a known race of *X. campestris* pv. *vesicatoria*, prior to any final conclusions about the phenotype. This is due to the possibility of recombination between the markers and the *Bs2* gene, which has been observed in the author's lab. Yoo et al (2001) identified flanking AFLP markers within 2.0 cM of another bacterial leaf spot resistance gene *Bs3*. They demonstrated the capacity for the markers to identify the resistance phenotype in both F_2 progeny and a resistant variety derived from the resistant parent- PI 271322. Recently, Jordan et al (2006) isolated a single BAC clone containing the *Bs3* gene, with markers at 0.01 cM distance. Cloning of this gene will allow comparison of its structure to the *Bs2* gene.

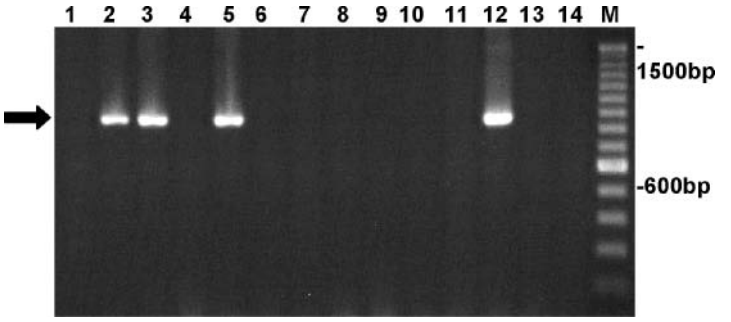

Fig. 2. Presence or absence of SCAR marker S45 (arrow) linked to the *Bs2* bacterial spot resistance gene in a pepper cross of J 201 x Sayula. #1 to #14= pepper plants from a cross of J 201 x Sayula and M=a 100-bp DNA marker ladder.

Other resistance genes have also been mapped in various pepper families. Lefebre and Palloix (1996) identified molecular markers associated with 13 putative QTL for resistance to *P. capsici* in an F_2 family segregating for resistance. Murphy et al (1998) used molecular markers to map the *pvr1* locus, and demonstrated that *pvr1* and *pvr3* are distinct gene loci, with different modes of action. Later, Kang et al (2005) cloned the *pvr1* gene and demonstrated that it was allelic to *pvr2*. Caranta et al (1999) identified a cleaved amplified polymorphic sequence (CAPS) marker tightly linked to the *pvr4* gene for PVY resistance in pepper. This marker was placed on the pepper genetic map by Grube et al (2000), who localized it to chromosome 10. This is also the chromosome found to contain the *Tsw* gene for resistance to tomato spotted wilt virus (Jahn et al, 2000). Lefebvre et al (1998) demonstrated linkage between the y locus for yellow mature fruit and the capsanthin-capsorubin synthase gene. Popovsky and Paran (2000) developed a codominant PCR marker from the CCS gene for marker assisted selection. The author's lab is developing PCR-based molecular markers linked to QTL associated with high flavonoid levels in peppers (Crosby, 2005; Author, unpublished data). Molecular markers are also utilized to determine taxonomic relationships in many plant species. Rodriguez et al (1999) used RAPD markers to assess genetic variation within different *Capsicum*

species and to differentiate between distinct species. This allowed classification of several accessions to the proper species, when previous morphological data led to misclassification. An integrated linkage map of pepper has been developed from the consolidated efforts of several research programs in the U.S., France and Israel (Paran et al., 2004). This map can be found at www.plbr.cornell.edu/psi/peppermap.pdf. It includes 2262 markers in thirteen linkage groups. The average marker density is roughly one every 0.8 cM. This is a good level of saturation, but due to the high number of families involved (6), more markers need to be added to reduce the linkage group number to match the 12 chromosomes of pepper, and reduce the number of large gaps in areas of linkage groups 7 and 8.

8.2 Genetic Transformation

Genetic transformation has been very difficult to achieve in pepper, compared to other Solanaceous crops. This is largely due to the difficulty in regenerating plants from transformed callus. Therefore, the utilization of cloned genes in pepper has lagged behind other crop plants. Binzel et al (1996) demonstrated successful regeneration of pepper from somatic embryos, which might allow greater success in developing transformed plants. Lim et al (1999) successfully transformed pepper with several genes, including the *Bar* gene for glyphosate resistance. They demonstrated stable, Mendelian inheritance of the gene in F_2 progeny of the transgenic plants. Shin et al (2002) successfully transformed hot pepper plants with tobacco stress-induced gene 1. This transgene induced expression of several pathogenesis–related genes, leading to resistance against CMV, PMMV, BLS and Phytophthora. In another study, resistance to CMV was observed in transgenic plants expressing satellite RNA from the virus (Kim et al., 1997). Park et al (2005) are currently transforming pepper with a vacuolar H+-PPase gene for inducing drought stress tolerance. These modern advances in molecular biology techniques are expediting gene discovery, but functional analysis is still crucial for practical applications. To plant breeders, this means collecting high quality phenotypic data, from multiple environments, prior to commercializing any new pepper cultivars. Marker-assisted selection and transgene expression will only be successful if appropriate phenotyping is carried out to confirm stable and effective gene expression. Recombination events, gene silencing and changes to gene promoters may lead to impaired expression, even when the desired gene sequence is present. The role of the breeder will remain crucial to confirm the desired function of key genes at the whole plant level.

9 Seed Production

The two distinct types of pepper cultivars, open-pollinated, and hybrid, require different means to produce commercial seed. Open-pollinated peppers, including land race types, are generally produced in field locations where climate permits. Isolation of one km or more is desirable to prevent any contamination by bees

carrying pepper pollen of other cultivars. In breeding programs or in small scale seed production, insect proof cages or greenhouses are often used to produce stock seed (Bosland, 1993; Pike-personal communication). It is wise to rogue any off-types as soon as they are obvious from plant or pod habit, so that their pollen does not cross-pollinate many flowers. Hybrid seed is produced mostly by manual cross-pollination of one elite inbred line by another. Much of the commercial seed industry produces F1 hybrid pepper seed in regions with extremely cheap labour costs, such as China and Thailand. This has lead to expensive seed for many pepper cultivars. Genic male-sterility is employed for production of a few hot pepper hybrid cultivars, after rouging fertile plants from the seed parent. Pollen may either be transferred by humans or by bees. In commercial hybrid seed production, the pollen parent often carries important dominant genes, rather than the seed parent. This protects proprietary germplasm from competitors, because a small percentage of self-pollinated seed allows identification of the female parents in most hybrid seed lots.

Seed is extracted from mature, coloured fruit from the best plants if some segregation is present, or from all plants if the cultivar is completely uniform. Mechanical harvesting equipment can be utilized for most hot pepper types. Different mechanical seed separators are employed for either wet or dried fruits (George, 1985). Seed is separated from the placenta, washed and cleaned with some disinfectant, such as sodium hypochlorite. The time and concentration will impact seed viability, so some experimentation should be conducted based on the cultivar and species. A solution of 3% for up to 20 minutes, will surface sterilize seeds, without much inhibition of germination (Khah and Passam, 1992). If PMMV is suspected of being present in the testa, a solution of 10% TSP may be used to decontaminate the seed. Some reduction of viability can be expected, usually less than 10%. After drying, seed is stored in a cool dry facility, preferably 4 ºC or lower. The author has found that pepper seed stored at -20 ºC maintains viability for more than 10 years. Cochran (1974) demonstrated that pepper seed size and weight impact germination and seedling uniformity. Seeds of 3.5 mm diameter and weight of 0.36-0.40 mg produced better transplants than seed less than 3.0 mm and 0.30 mg. Specific gravity tables are used by commercial seed companies to eliminate seed of insufficient mass, thereby assuring better uniformity of germination (Vaughan et al., 1968). Most commercial pepper seed containers are labelled with the germination percentages. Pepper seed may sometimes exhibit dormancy, which may inhibit germination. This may be overcome by treatment with gibberillic acid or potassium nitrate (Bosland and Votava, 2000; Watkins, 1985). Conflicting results have been reported on the effects of seed priming on pepper germination rates and stand establishment (Rivas et al., 1984). It is not widely employed by the industry in the U.S. Most hybrid pepper seed is coated with a fungicide such as Thiram, to prevent damping off, caused by various soil fungi.

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Tomato

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1 Introduction

The tomato belongs to the Solanaceae family along with other economically important crops such as pepper, eggplant and potato. The tomato was classified by Miller (1754) as *Lycopersicon esculentum* and renamed by Child (1990) and Peralta and Spooner (2006) as *Solanum lycopersicum.* Tomato is a diploid species with 2n = 2x = 24 chromosomes. The tomato genome is composed of approximately 950 Mb of DNA, more than 75% of which is heterochromatin and largely devoid of genes.

The tomato was cultivated and consumed in Mexico well before the arrival of the Spanish. Its introduction and diffusion in Europe were accompanied by a transdomestication which developed different types associated with new uses and growing systems. As a result, an increase in yield and fruit quality relative to the new uses took place during this period. More recently, the exploitation of heterosis, the development of cultivars adapted to processing, and the exploitation of extraspecific variability, especially of that related to resistance to biotic and abiotic stresses, have given rise to the development of current varieties. The success of this crop is evident. The tomato has passed from uncertain acceptance to its arrival in Europe to occupying first place among horticultural crops at the present. Worldwide production of tomatoes reached 122 million tonnes in 2005 (FAOSTAT data, 2005). Asia is by far the continent with the greatest production, producing 50% of the total, with Europe ranking as the second (17.5 %), followed by Central and North America (12.3 %), Africa (11.7 %), Latin America and the Caribbean (7.8 %) and Oceania (0.05 %). China is the main producer of tomato, followed by the US, Turkey, India and Italy.

In spite of the considerable improvement obtained to date, the application of new biotechnological tools offers the promise to address new objectives technically unattainable until now. One new biotechnology that may prove to have the greatest

impact is molecular markers, which allow, among other things, marker-assisted selection. The identification, isolation and cloning of specific genes has permitted the development of transgenic crops. The commercial importance of these crops is still limited, due to the rejection by certain sectors of consumers and because in many cases the economic advantages of this type of crops are not notably greater than those of crops obtained by other breeding techniques. Genomic tools currently being developed in various genomic projects are facilitating the advance towards many objectives with new methodologies. Two examples are large-scale gene expression profiling and the TILLING or EcoTILLING techniques used in searching for alleles of interest. Genomics offers the possibility of a better understanding of genome organization, breeding guided by genetic and physical maps, and an understanding of the regulatory architecture underlying biochemical pathways in secondary metabolism and trait expression. All these new possibilities are being applied through two approaches: a) the analysis of each individual component of complex characters and b) the identification of QTLs for the study of quantitative characters highly influenced by environment. Organoleptic and nutritional quality of fruits and the resistance to biotic and abiotic stresses, when the only known source of resistance are polygenic, are the great breeding objectives these new methodologies are currently dealing with. The comparison of the tomato genome to those of other horticultural crops such as pepper, eggplant and potato, permitted by the availability of high-density genetic maps and sequenced ESTs, has resulted in findings of great potential interest.

The bibliography for this crop is very extensive, with the contributions of Atherton and Rudich (1986), Kalloo (1991) and Nuez (1995) being some of the most prominent. This review will focus mainly on the major breeding achievements in tomato, the current breeding objectives and the applications of new biotechnologies in tomato breeding.

2 Origin, Domestication and Diffusion

The centre of origin of the genus *Solanum* section *Lycopersicum* (formerly genus *Lycopersicon*) is the Andean region that includes parts of Colombia, Ecuador, Peru, Bolivia and Chile. All tomato wild relatives are native to this area (Rick, 1973; Taylor, 1986).

Until the arrival of the Spanish explorers to America, the tomato was cultivated in the small vegetable orchards of the Mesoamerican area and was of little economic importance. It was one more of the weeds of the "milpas" (small family orchards). The European chroniclers made few references to it, and sometimes misinterpreted certain quotes that contain the word *tomatl* as referencing the tomato, when they were really referring to another species. This may have produced an overestimation of the real importance of the tomato in pre-Columbian times. The word *tomate*, introduced into the Spanish language in 1532 (Corominas, 1990), comes from the Nahuatl word *tomatl* and, according to Fray Bernardino of Sahagún (1577) in his book "Historia general de las cosas de la Nueva España" (written between 1548 and 1578, Sahagún, 1988), it was applied in a general manner to plants bearing spherical

fruits or berries, with many seeds and juicy flesh (Williams, 1990; Montes y Aguirre, 1992). In order to indicate the particular species, a qualifying prefix was used (Table 1). When the expression *tomatl* or tomato was used, it referred to any one of these species or to the most popular at the time, the "milpero", or husk-tomato (*Physalis philadelphica*). As a result of the use of the word "tomato" as a generic voice, it is not always easy to interpret the concrete species to which the chronicler was referring. However, it seems certain that in pre-Columbian Mexico, the husk-tomato (*Physalis philadelphica*) was much more popular than the *jitomate* (*Solanum lycopersicum*).

Table 1. Nahuatl words related to *tomatl* cited in the "Historia general de las cosas de Nueva España" of Fray Bernardino of Sahagún (1577, ed. 1988).

"Nahuatl" word	**Translation to Spanish (English)**	**Species**
miltomatl	tomate de la milpa (tomato of the "milpas")	*Physalis philadelphica*
tepetomatl	tomate del cerro (tomato of the hill)	*Physalis* (?)
coztomatl	tomate amarillo (yellow tomato)	*Physalis costomatl*
xitomatl	tomate rojo, jitomate (red tomato)	*Solanum lycopersicum*
coyotomatl	tomate de coyote (coyote tomato)	*Vitex mollis*
xaltomatl	tomate de la arena (sand tomato)	*Saracha jitomata*
tecomatl	tecomate	Medicinal Plant (?)

There still exist unknown aspects with respect to the origin and domestication of the cultivated tomato. Nevertheless, there are some points about which we have a reasonable degree of certainty (Rick, 1976, 1978).

-The cultivated tomato had its origin in the New World. It was unknown in Europe and the rest of the Old World before the discovery of the Americas.

-The cultivated tomato had reached a certain level of domestication before being taken to Europe and Asia.

-The most likely ancestor of tomato is the wild cherry tomato, formerly *Lycopersicon esculentum* var. *cerasiforme* (Dun.) Gray. This species is spontaneous throughout tropical and subtropical America and has spread throughout the tropics of the Old World.

The place where the domestication took place has been a controversial topic. Some authors state that domestication took place in Peru, as it was introduced in Italy with the names of *Mala peruviana* or *Pomi del Perú* (Candolle, 1883; Luckwill, 1943). However, it is known that it was consumed only sporadically in the Incan Empire and no remains of tomato have been found in archaeological sites in the Andean region where remains of other crops have been found. Therefore, no clear evidence supports this hypothesis.

Most of the evidence supports Central American domestication, with Mexico as the probable region of domestication. Linguistic, historical and genetic evidence supports this hypothesis (Esquinas-Alcázar and Nuez, 1995):

-Isozymic variation studies indicate that the European cultivars show a greater similarity to the primitive cultivars and lines of the *cerasiforme* variety from Mexico

and Central America than to the ones which came from the Andean region (Rick, 1971; Rick et al., 1974; Rick and Fobes, 1975).

-The tomato was totally integrated in the "Azteca" culture in the 16th century. "Lo cultivaban, lo vendían y lo consumían" ("They cultivated, sold and consumed it"), pointed out the writer Fray Bernardino of Sahagún talking about the Mexican markets in its book "Historia de las cosas de Nueva España" (Sahagún 1988). Other historical references that describe the presence of the tomato in the Mexican markets, as well as their local use in typical dishes, are those of Francisco Hernandez (1570, ed. 1790) in his book "Historia de las Plantas de Nueva España" and José de Acosta (1590) in his "Historia natural y moral de las Indias". However, the same is not true of the Andean region. In this area, there could have been at the most an incidental consumption of non-cultivated forms.

-The tomato did not have any known name in *quechua, aymara* or any other Andean language, whereas the word *tomatl* existed in *nahuatl,* the native language of Mexico, and described plants bearing globous and juicy fruits. This word was modified with prefixes, *xitomatl* ("red tomato") being the word employed to name the tomato (*S. lycopersicum*) (Table 1, Sauer, 1993; Gould, 1983).

-Remains of this plant have not been found in archaeological sites in the Andean region, whereas parts of most other cultivated native plants, like the potato, the pepino or the pepper have been found (Rick. 1978; Sauer, 1993).

One of the most important consequences of the domestication of the tomato was the change from exerted to inserted stigmas, the change from partial allogamy to strict autogamy and increased fruit size.

In spite of being domesticated in Mexico, the tomato was known and accepted in some zones of the Old World before it was in the rest of the American continent (Table 2). The establishment of commercial routes and colonies contributed to its diffusion everywhere. There are several routes of expansion (Figure 1., Esquinas-Alcázar and Nuez, 1995):

1.-Clear evidence of the introduction of tomato in Europe began appearing in the herbals of the mid-16th century. The first record, in 1544 in Italy by Matthiolus, was of a yellow-fruited form under the name *pomo d´oro*, a name which survives even today. Early European sources noted the fruit was edible, but its spread though Europe was initially as a garden curiosity, not as a food plant, and there were rumours that it was dangerous. Finally, by the late 1700s, tomatoes were being grown and eaten in abundance in Italy and the Iberian Peninsula, although Northern Europeans were afraid of them and its adoption was slow.

2.-It is known that the tomato was present in Africa at the end of the 16th century, specifically in Egypt and Tunisia. The role of Turkish traders was important with regard to its diffusion through the Mediterranean Basin and Near East, as was the role of the Portuguese spice traders in the enclaves of Mozambique and Angola (Villareal, 1980; Esquinas-Alcázar and Nuez, 1995).

3.-The introduction of the tomato in Asia was probably carried out from the Philippines, through the galleon trade maintained with Spain as of the 16th century. The maritime trade with the islands and nearby countries such as China, Japan and India could have contributed to its later diffusion throughout the continent. Another possible route of diffusion might have been through the Portuguese commercial

enclaves in Asia. It was introduced in Korea in the 17th century, whereas in Japan and India its culture was not known until the 18th century. The introduction in China was delayed, and the tomato was of little to no commercial importance until the 19th and 20th centuries (Esquinas-Alcazar and Nuez, 1995).

Table 2. First historical references to the consumption and cultivation of the tomato in the Old and New Worlds from the 16th century.

Country	Author	Use	Source
Italy	Pier Andrea Mattioli	Consumption, 1544	Mattioli, 1544
Spain	Gregorio de los Rios	Ornamental, 1592	Los Rios, 1592
	Hamilton	Consumption (purchase list of the "Hospital de la Sangre", Seville), 1608	Hamilton, 1976
	José Quer	Culture and consumption, 1762	Quer, 1762-84
	Claudio and Esteban Boutelou	Culture and consumption, 1801	Boutelou, C and E, 1801
United Kingdom	Gérard	Presence, 1597	Gérard, 1597
France	Catalogue des Graines Vendus Andrieux Vilmorin	Ornamental, 1760 Horticultural crop, 1785	Fournier, 1947-1948
United States of America	William Salmon Thomas Jefferson	Presence, 1710 Culture, 1782	Rick, 1978
Santo Domingo	Fernando de Araújo y Rivera	Culture, 1699	Rodríguez-Demorizi, 1942
Peru	Hipólito Ruíz	Culture, 1777-1788	Ruiz, 1952
Ecuador	Juan de Velasco	Culture, 1789	Velasco, 1927
Bolivia	Antonio Vázquez de Espinosa	Culture, 17th c.	Vazquez de Espinosa, 1948
Panama	Berthold Seeman	Culture, 1853	Seeman, 1853

4.-The crop spread into the New Word by way of the Spanish and Portuguese colonists. References to the consumption and cultivation of this horticultural crop on Caribbean islands including the Antilles and in other Latin American countries have been found dating to the 17th and 18th centuries (Table 2). Direct introduction of material from Mexico to the United States may have occurred, although references in this regard have not been found. Its cultivation was already known on the eastern North American coast in the 18th century, where it was introduced by European colonists. The earliest record of tomato cultivation in California is in San Diego around 1850.

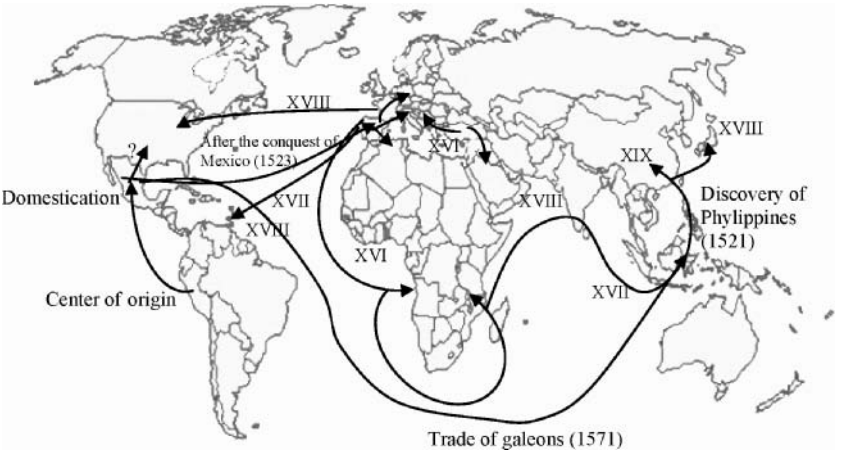

Fig. 1. Possible spreading routes of the tomato beginning in the 16th century (Based on Esquinas-Alcázar and Nuez, 1995).

3 Varietal Groups

The tomato has experienced a great diversification due to the great diversity of uses and its adaptation to different cropping systems. As a result of this process hundreds of varieties of tomatoes are now available.

Tomatoes can be consumed raw, boiled, stewed, as a sauce or in combination with other foods. They can be used as an ingredient in the kitchen or can be commercially processed whole or as a paste, juice, powder, and so on. Tomatoes can be divided into two groups according to their use: for fresh consumption and for processing. Within each of these groups, specific cropping systems exist that require adapted varietal types. Varieties for fresh consumption are cultivated in greenhouses and in the open air, while varieties for processing are only cultivated in the open air.

3.1 Tomato Varieties for Fresh Consumption

Tomatoes for fresh consumption obtain high prices in the market and the new hybrid varieties are high yielding. This high profitability permits growers a type of culture where the infrastructure investments are very expensive. The varietal types used in this type of cultivation are adapted, in many cases, to greenhouses and long periods of cultivation and harvest, which allows a monetary return to growers that compensates the investment made. From the cultivation by growers to the consumer there are several agents involved in the producer-consumer chain. In the case of tomato for fresh consumption, this chain includes the following steps: seed producer, grower, carrier from the field, storekeeper, and carrier to the retailer, retailer and

consumer. Each of these agents has specific requirements demanding specific characteristics of the varieties.

The characteristics required for a variety for fresh consumption are open growth habit, high yield, earliness, external quality of fruits (shape, colour, homogeneity), internal quality of fruits (flavour, sweetness, juiciness), long shelf life, adaptation to growing systems and resistance to biotic and abiotic stresses. If the cultivation is conducted in greenhouse, other characteristics are required, such as adaptation to long harvesting periods, not excessively compact plants and adaptation to low temperature and light intensity. Recently, the cultivation of cluster tomato types, also called truss tomatoes or on-the-vine tomatoes, has increased. Cluster types require some additional attributes such as uniformity of size and ripening within the cluster and maintaining a fresh green calyx and vine after harvest. There exist several varietal types, each with specific characteristics of plant and fruit. Table 3 and Figure 2 show the different types grouped according to the size and ribbing of the fruits and growth habit.

Table 3. Varietal types of tomato for fresh consumption.

Fruit size	Fruit ribbing	Growth habit	Type
Big fruits Caliber G and GG > 67 mm	Smooth or slight	Indeterminate or determinate	BEEFSTEAK
	Medium or strong	Determinate or indetederminate	MARMANDE
Medium sized fruits Caliber: M 57 - 67 mm	Smooth or slight	Indeterminate or determinate	VEMONE (round or slightly flattened)
		Indeterminate	PEPPER-SHAPED
Small fruits Caliber MM 47 – 57 mm	Smooth	Indeterminate	MONEYMAKER AND CANARY
			HANGING BASKET TOMATO
Small fruits Caliber MMM < 47 mm	Smooth	Indeterminate	COCKTAIL (round and pear-shaped)
Very small fruits < 30 g	Smooth	Indeterminate	CHERRY (edible and ornamental)

BEEFSTEAK: Determinate or indeterminate growth habit, flattened or round, multi-locular fruits with or without green shoulders.

MARMANDE: Determinate or indeterminate growth habit, large, irregularly shaped, multi-locular, highly ribbed fruits, frequently with green shoulders.

VEMONE: Mostly indeterminate growth habit, slightly flattened or spherical in shape, two- to three-locule fruits, very vigorous plants. There are varieties for cluster or single fruit harvesting.

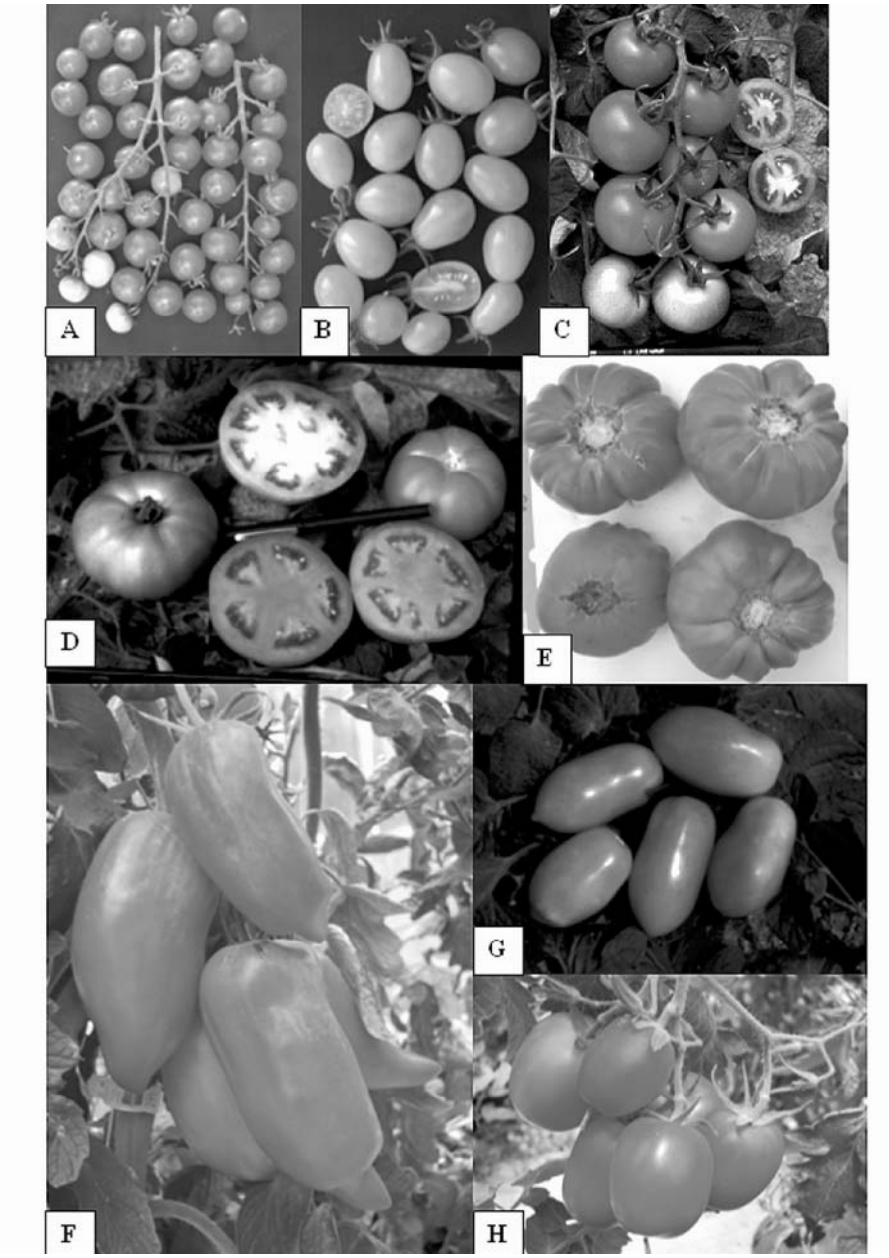

Fig. 2. Different fruit types of tomato. A) Cherry, B) Cocktail pear-shaped, C) Canary for cluster harvesting, D) Beefsteak, E) Marmande, F) Pepper, G) tomato for processing, long pear shaped and H) tomato for processing, square shaped.

PEPPER-SHAPED: Indeterminate growth habit, long pepper-shaped fruits, compact and fleshy fruits, used for fresh or processing consumption. Local use.

MONEYMAKER AND CANARY: Indeterminate growth habit, round, smooth fruits, without green shoulders.

HANGING BASKET TOMATO: Dwarf growth habit, very small round fruits, various colours of fruits and foliage, ornamental and human consumption uses.

COCKTAIL: Indeterminate growth habit, round, plumb or pear shaped fruits, between 30 and 50 g.

CHERRY: Indeterminate growth habit, very small round or pear-shaped fruits, between 10 and 30 g, long clusters. There are varieties for cluster or single fruit harvesting.

Most of the described types have red mature fruits. However, there exists a great variability of colours which include red, orange, yellow, whitish or black, with or without spots, with or without green shoulders. Both the final colour and the colour at breaking are important (Figure 3).

Most varietal types, especially those cultivated in greenhouse, have introgressed a high number of genes of resistance mainly to fungi and viruses (Laterrot, 2000). The following are the genes of resistance most commonly used by seed companies. Genes of resistance to viruses: *Tm-2*^{2}, *Sw-5* y *Ty-1*. Genes of resistance to fungi: *Ve, I, I-2, Fol 1,2, Frl, Asc, Cf*-series, *Sm, Ph-2* and *Lt* (In Table 7 the pathogens to which the named genes confer resistance are indicated). In the case of bacteria, the *Pto* gene is the most used. Finally, one of the first genes introgressed into tomato was the *Mi* gene, which confers resistance to *Meloidogyne incognita*. Other genes conferring resistance to other species of nematode are also currently used.

3.2 Tomato Varieties for Processing

Tomatoes for processing differ from tomatoes for fresh consumption in many aspects. The fresh product is bought by the industrial and does not obtain the prices that tomatoes for fresh consumption do. To be profitable, the production costs must be drastically reduced, turning the tomato from a horticultural crop into an industrial crop with mechanized harvesting. This requires the plant and fruit to be adapted to this type of harvest and therefore the fruits possess some specific characteristics to facilitate a high profit in the industrial process. The producer-consumer chain includes industrial processing in this case. Some requirements are common to all the agents of the chain, but many are specific. The characteristics needed in the tomato for processing are compact growth habit of the plant, grouped flowering and ripening, presence of the recessive *jointless* gene which facilitates the detachment of the fruit without the peduncle, homogeneity of fruit shape and size, high consistence, resistance to cracking, lack of scar at the point of insertion with the calyx, lack of puffiness, flexible skin to facilitate peeling, thick and firm pericarp, round, smooth plumb- or pear-shaped fruits and red, uniform colour. The fruits must also have certain other characteristics related to processing quality: high viscosity and dry extract, pH values between 4.2 and 4.4 and high values of total soluble acids. There are two main types of processed products: tomato concentrate and whole peeled

tomato. Shape requirements are stricter for whole peeled tomato, small-sized, pear, pear-elongated, pear-oval or cylindric-shaped fruits being more suitable, as these more stylized shapes facilitate peeling.

Fig. 3. Variability in fruit colour.

Of all the components of the producer-consumer chain, perhaps the most important is the processor. To exemplify, Table 4 specifies some of the requirements of the industrial, grower and consumer, which imply some specific breeding objectives to breeders (Table 4).

In tomato for processing two main varietal types can be found, depending on the use: varieties for paste and varieties for whole peeled. The differences in requirements are mainly in shape and size of the fruit. In the varieties for whole

peeled fruits have to be pear, long pear, oval or cylindrical shaped because these shapes facilitate the peeling. The size can vary between 60 and 100 g. or even smaller for whole canned. Varieties for paste are less restrictive if shape and size of fruits. Squared, oval and round fruits are admitted and the size can vary between 60 and 130 g.

Table 4. Necessities of the processor, growers and consumers and some associated breeding objectives in tomato for processing (Modified from Schroeder, 1993).

Requests	**Breeding objectives**
Processor	
High % usable fruits	Fruit firmness, resistance to cracking Ripe conservation capacity Resistance to diseases Good foliar cover
High factory yield for each type of processed product: paste, peeled (canned whole, sliced, crushed, halved, blended), ketchup and sauces, juices and soups, dehydrated	Soluble solid content, viscosity, pectins, Uniform shape and size Soluble solids, acidity, dry matter
Flexibility in factory timing: early start-up , main season, late season	Early maturity, cold ability, heat set ability, disease resistance
Grower	
High yield	Adequate number of fruits and fruit weight
Low production costs: low pesticide use, easy handling of plant	Resistance to pests and diseases, adequate growth habit and branching of the plants, varieties adapted to mechanical harvest
Flexibility: diverse cultivation cycles and periods, varieties with multiple uses	Early and late varieties, varieties with multiple uses
Consumer	
Nutritional value	Increase in vitamin content, energetic value
Culinary appeal: colour, texture, flavour	Increase in carotenoid content, especially lycopene, soluble solids content

4 Genetic Resources

The first source of variability used for tomato breeding was intraspecific variability. This allowed the diversification of new types adapted to the new uses of tomato. Genes such as the *sp* (*self pruning*) gene, which endows the determinate plant growth habit, and the *nor* (*non-ripening*) and *rin* (*ripening inhibitor*) genes, which determine dramatic alterations in the ripening process, come from *S. lycopersicum.* These genes all lead to qualitative advances in tomato breeding. However, this variability was not

sufficient to solve the problems originated by diseases nor to improve some aspects of the organoleptic and nutritional quality of the fruits. This led to the search for new sources of variation in wild species. Wild relatives of tomato have been a valuable source of genes of interest for tomato breeding (Table 5).

Table 5. Characteristics of interest of wild tomato relatives in tomato breeding (modified from Esquinas-Alcazar and Nuez, 1995).

Species	Characteristic of interest
S. lycopersicum var. *cerasiforme* L.	Tolerance to humidity, resistance to fungi and root rot
S. cheesmaniae (L. Riley) Fosberg and *S. galapagense* S. Darwin & Peralta	Tolerance to salinity, *jointless* gene and thick pericarp
S. pimpinellifolium L.	Colour, characteristics of quality, resistance to diseases
S. chmielewskii (C.M. Rick, Kesicki, Fobes & M. Holle) D.M. Spooner, G.J. Anderson & R.K. Jansen	High sugar content
S. neorickii D.M. Spooner, G.J. Anderson & R.K. Jansen	Resistance to bacteria
S. pennellii Correll	Resistance to drought
S. habrochaites S. Knapp & D. M. Spooner	Tolerance to cold and chilling, resistance to insects and diseases
S. chilense (Dunal) Reiche	Resistance to drought and diseases
Complex peruvianum*: S. peruvianum* (L.), *S. arcanum* (Peralta), *S. corneliomuelleri* (J.F. Macbr.), *S. huaylasense* (Peralta & S. Knaap)	Resistance to viral, fungal and bacterial diseases

Interspecific hybridization has facilitated the exploitation of genetic resources in tomato breeding. The incompatibility barriers between some wild species and the tomato have been overcomed by the rescue of immature embryos and their in vitro cultivation (see "Breeding methods and techniques"). The crossability relationships of the wild relatives of tomato are given in Figure 4.

Important germplasm collections of tomato and wild relatives exist in many countries. The C.M. Rick Tomato Genetics Resource Center (TGRC), located at the University of California at Davis, is one of the best collections, and includes wild relatives, monogenic mutants and miscellaneous genetic stocks of tomato. Table 6 shows the most important collections of tomato germplasm, which hold more than 1000 accessions from all over the world.

Most of the accessions are tomato landraces and wild species. However, some collections include interspecific hybrids (AVRDC), collections of natural or induced mutants and different types of populations, such as introgression lines from different wild species (TGRC, The Volcani Center).

In Europe, Solanaceae genetic resources are coordinated by the European Cooperative Program for Crop Genetic Resources Networks (ECP/GR). The Solanaceae Working Group, which includes tomato, pepper, eggplant and other less

important crops, was established in 2001. The activity of this group in the coordination of genetic resources in Europe is available on the internet (http://www.ecpgr.cgiar.org/Workgroups/solanaceae/solanaceae.htm).

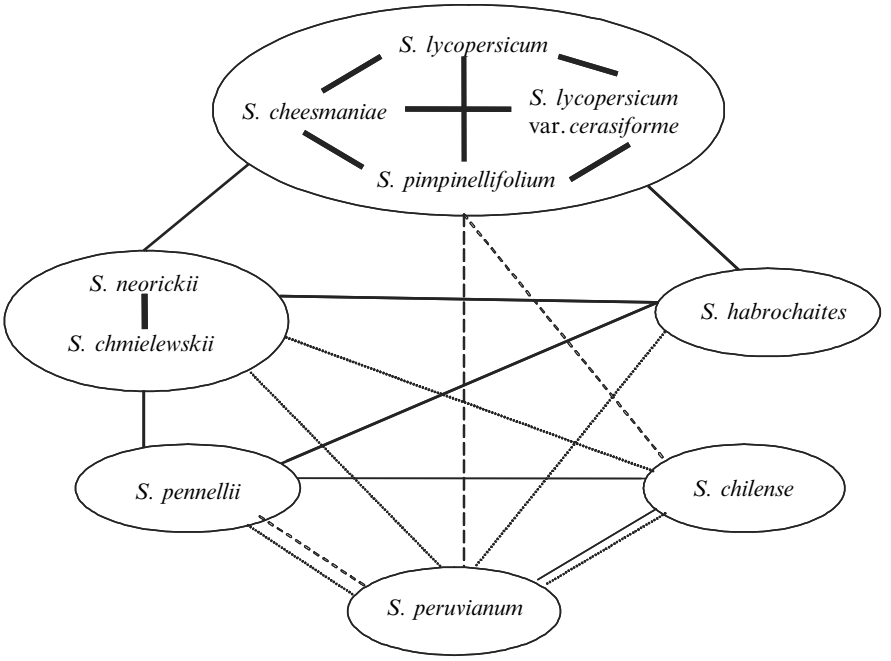

Fig. 4. Diagram of crossability of tomato and its wild relatives (modified from Stevens and Rick, 1986).

Wild tomatoes are native to western South America and are distributed from northern Ecuador, through Peru to northern Chile and in the Galapagos Islands. Wild tomato species grow in a variety of habitats, from near sea level along the arid Pacific coast to over 3300 m in the numerous valleys of the western side of the Andes (Rick, 1973; Taylor, 1986).

The taxonomic status of wild tomatoes within Solanaceae has been controversial since the eighteen century. In 1753, Linnaeus placed the tomato in *Solanum,* while Miller, a contemporary of Linnaeus, classified tomatoes in a new genus, *Lycopersicon*. In the genus *Lycopersicon* the stamens forms a solid tube cone shaped with long connective tissue. Anther dehiscence is introrsely longitudinal which differentiate this genus from the genus *Solanum* in which the dehiscence of the anthers is poral.

The opinion of Miller has been accepted by the majority of botanists. Writings on the history of tomato taxonomy are abundant and are reviewed extensively in Taylor et al. (1986), Hunziker (1979) and D'Arcy (1979), and also in Peralta and Spooner (2000, 2005). However, the genera *Lycopersicon* and *Solanum* are very close. More specifically, tomatoes, potatoes and pepinos (*S. muricatum* Ait.) are members of the same monophyletic group (Lester, 1991; Spooner et al., 1993). This has led to a change in the name of tomato by certain authors who include it in the *Solanum* genus Section *Lycopersicon* (Child, 1990; Marshall et al., 2001; Peralta and Spooner 2001; Peralta et al., 2005**;** Spooner et al., 2005) (Figure 5). The major changes have occurred in the former *Lycopersicon peruvianum* species, which has been divided into four new species (Peralta et al., 2005). However, there exist a series of intermediate forms between them, constituting a complex situation where some specimens are quite difficult to classify.

Table 6. Tomato collections holding more than 900 accessions.

Country	Institute	Number of species	Number of accessions
Australia	Australian Tropical Crops & Forages Genetic Resources Centre, Queensland	8	1116
Azerbaijan	Genetic Resources Institute, Baku	1	2800
Brazil	Centro Nacional de Pesquisa de Hortaliças (CNPH), EMBRAPA, Brasilia	1	2070
Bulgaria	Institute of Plant Genetic Resources, Sadovo, Bulgaria	8	1134
Canada	Horticultural Experiment Station, Ontario	4	1070
China	Institute of Crop Science (CAAS), Beijing	1	1942
Colombia	Corporacion Colombiana de Investigacion Agropecuaria – CORPOICA, Palmira	1	2018
Czech Republic	Faculty of Science, Palacky University, Olomouc	5	1613
France	Unité Expérimentale d'Angers GEVES	1	1254
France	Station d'Amélioration des Plantes Maraîchères, INRA Avignon, Montfavet	13	1360
Germany	Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben	3	2965
Hungary	Institute for Agrobotany, Tápiószele	5	2043
Israel	The Volcani Center, Hebrew University, Jerusalem	1	3076

Japan	National Institute of Agrobiological Sciences, Tsukuba	7	1217
The Netherlands	Center for Genetic Resources, Wageningen, The Netherlands	11	1700
Peru	Universidad Nacional Agraria La Molina, Lima	7	936
Philippines	National Plant Genetic Resources Laboratory, IPB/UPLB, Laguna	6	4793
Poland	Research Institute of Vegetable Crops, Skierniewice	1	917
Russian Federation	Vavilov Institute of Plant Industry,VIR, St. Petersburg, Russian Federation	12	7250
Serbia and Montenegro	Institute of Field and Vegetable Crops, Novi Sad	1	1030
Spain	Centro de Recursos Fitogeneticos, INIA, Madrid	1	1267
Spain	Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Valencia, Spain	12	3917
Spain	Banco de Germoplasma de Hortícolas, Zaragoza	5	1380
Taiwan	Asian Vegetable Research and Development Centre, Taiwan	9	7235
Ukraine	Institute of Vegetable and Melon Production, Selektsijne	1	2433
US	C.M. Rick Tomato Genetic Resources Center Davis	10	3157
US	Campbell Institute for Agric. Res. Campbell Soup Company, Camden	1	4572
US	National Center for Genetic Resources Preservation (NCGRP), USDA-ARS, Fort Collins, Colorado	3	1482
US	Cornell University, Jordan Hall, NYS AES, Geneva	2	4850
US	Northeast Regional Plant Introduction Station PGRU, USDA-ARS, Cornell University, Geneva	10	5804

Source: Daunay *et al*., 2003; http://www.ipgri.cgiar.org/germplasm/dbintro.htm

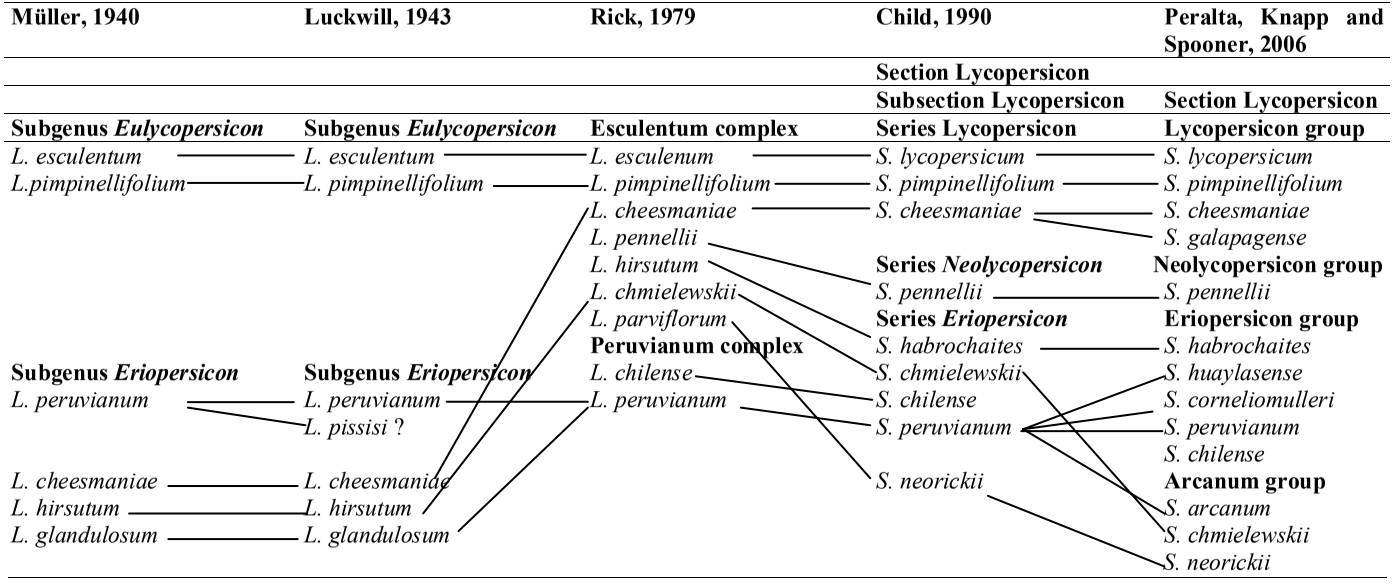

Fig. 5. Comparison of classification of *Solanum* sect. *Lycopersicon.* The lines connect synonymous taxa (Modified from Peralta and Spooner, 2006).

5 Major Breeding Achievements

The first breeding achievements were obtained during the same domestication process. The selection conducted on the first specimens that arrived from Peru gave rise to a certain diversity of types and colours in Mexico, before the tomato's transport to Europe. After its introduction in Europe, a new domestication, the transdomestication, took place. This one consisted of the hybridization of already existing types and a selection which searched for new types adapted to the new uses and growing systems. Yield increase has been one of the most important objectives of breeding programs, and important achievements have been made. Another great success in tomato breeding was the development of cultivars for processing in the United States, concretely in California. Other achievements have been obtained through:

-the exploitation of wild relatives in breeding thanks to the development of techniques that allowed crossability barriers to be overcome; this permitted the introgression of a high number of genes, mainly genes of resistance to diseases.

-the exploitation of heterosis and the development of hybrids which allowed important yield increases.

The need for cultivars with an increased conservation period after harvest has lead to the development of "long shelf life" cultivars. They have had great success and have been widely cultivated. Another area in which extensive research has been done, although without spectacular results, has been in the development of cultivars adapted to abiotic stresses, such as salinity, drought and high and low temperatures.

5.1 Breeding for Yield

Yield increases have been obtained directly, by increasing the harvest index, and also indirectly, by improving the potential production through the resistance or tolerance to biotic or abiotic stresses. The harvest index has been increasing until reaching, in some cultivars for processing, values of ½ and even up to 3/5 (fruits/vegetative part), which are considered optimal. In tomato for fresh consumption, these values are lower, but they tend to come near to those of industry. This success is due to the correct combination of the different components of yield: truss number, number of flowers per truss, number of fruit set and fruit weight. Thus, varieties that produce up to 15 kg/m^2 and with a high percentage of commercial yields have been developed.

5.2 Breeding of Tomato for Processing

Breeding for mechanized harvesting was started almost simultaneously by Hanna (California Univ., Davis, USA) and by Senyushkin (Mayak Breeding Station, the Russian Federation) in 1943. The first objectives set by breeders in order to obtain a cultivar suitable for mechanical harvest were greater firmness, to protect the fruit from machine damage, and a plant type with a very short fruit set period to simultaneously produce a high percentage of ripe fruits.

VR145 (resistant to verticillium wilt and fusarium wilt) was the first cultivar for machine harvest to achieve widespread use, reaching a record in harvested area in

California in 1962. Selections made from this cultivar were the most cultivated during more than 10 years.

Many cultivars suitable for mechanical harvesting were later released in many countries with improvements in synchronous maturity, resistance to overripening and to bruising, fruit firmness, marketable yield, resistance to cracking and jointless pedicel.

This drastically changed the technology of tomato cultivation and harvesting and contributed considerably to increased tomato production.

More recently, over the past 20 years, an important advance in processing tomatoes has been the replacement of open pollinated varieties with F1 hybrids. In some areas, these are now grown on close to 100% of the acreage, for instance in California and Israel. Approximately 27% to 38% of the total yield improvements made over the past 20 years in California and Israel have been attributed to the use of hybrid cultivars (Grandillo et al., 1999).

5.3 Exploitation of Wild relatives in Tomato Breeding: Introgression of Genes of Resistance

The development of in vitro culture techniques facilitated the hybridization of tomato and its sexually incompatible wild relatives, and consequently also facilitated the introgression of genes of resistance. This allowed the exploitation of all the potential of the wild relatives of tomato. Some genes of resistance introgressed in the modern cultivars are indicated in Table 7.

The development of hybrid cultivars with up to a dozen genes of resistance has allowed the cultivation of tomatoes in greatly diverse growing systems all over the world.

5.4 Development of Hybrid Varieties

The first commercial hybrids appeared in the market in the mid-twentieth century, although their valued performance was recognized a century and a quarter ago. Heterosis has been found in characters related to yield and adaptation to adverse conditions. Thus, heterosis has been shown for characteristics such as plant height, earliness, total yield, resistance attributes, uniformity and resistance to extreme conditions. Heterosis has also been found for characteristics related to fruit quality, for instance pericarp thickness, total soluble solids content and ascorbic acid content (Akhilesh and Gulshan, 2004).

In addition to the use of hybrids for their heterosis, the fact that the genes are heterozygous can also represent an advantage. Thus, the *rin* (*ripening inhibitor*) gene in a homozygous state completely avoids fruit ripening. In the heterozygous state, the fruits of plants bearing the *rin* and *alc* (*Alcobaça*) genes have fruits with normal pigmentation, with the ripening being delayed and the commercial life being increased (Nguyen *et al*., 1991; Mutschler *et al*., 1992).

Another example would be the R-genes with resistance to diseases. Many of these genes are dominant. Their effect appears in hybrids provided they are in one of the parentals. Some of these genes confer additional advantages if they are in a

heterozygous state. Thus, the genes *Mi, Tm* and Tm^2 had unfavourable effects in the first hybrids, such as a decrease in pollen fertility, poor fruit set, necrosis at high temperatures or a lack of firmness in inbreds (Soost, 1959; Laterrot, 1973; Philouze, 1976; Lapushner and Frankel, 1979). These effects, probably due to chromosomic effects linked to the gene of resistance present in the wild donor species, disappear in the hybrids, in which the genes are in a heterozygous state. This is the case of the *I-3* gene, which confers resistance to race 3 of *Fusarium oxysporum* f. sp. *lycopersici,* in which case the heterozygous plants had a higher percentage of fruits with a commercial size. A region of chromosome 7, close to the gene of resistance *I-3,* is thought to affect the size of the fruit, and therefore the use of this gene in heterozygosis is more appropriate (Scott, 1999).

Today, nearly all the tomato cultivars for the fresh market as well as an increasing number of cultivars for processing are hybrids. This type of cultivar provides a physical patent, which is very valuable for breeders.

Table 7. Genes of resistance to the most important diseases of tomato.

Disease	**Pathogen**	**Gene of resistance**	**Source**	**Reference of genetic control**
Fungi				
Verticillium wilt	*Verticillium dahliae*	*Ve*	*S. pimpinellifolium*	Cannon and Waddoups,1952
Fusarium wilt	*Fusarium oxysporum* f. sp. *lycopersici*			
	--pathotype 0	*I*	*S. pimpinellifolium*	Kesavan and
	--pathotype 1	*I-2*	*S. pipimnellifolium*	Choudhuri, 1977
	--pathotype 2	*I-3*	*S. pennelli*	Alexander and Hoover, 1955 Scott and Jones, 1989
Alternaria stem canker	*Alternaria alternata* f. sp. *lycopersici*	*Asc*	*S. lycopersicum*	Clouse and Gilchrist, 1987
Grey leaf spot	*Stemphyllium* spp.	*Sm*	*S. pimpinellifolium*	Andrus et al., 1942
Leaf mould	*Fulvia fulva* (*Cladosporium fulvum*)	*Cf* (1 to 24)	*S. pimpinellifolium* *S. lycopersicoides* *S. habrochaites* *S. peruvianmum*	Kerr et al., 1971
Powdery mildew	*Leveillula taurica*	*Lv*	*S. chilense*	Stamova and Yordanov, 1990
	Oidium neolycopersici	*Ol-1* *Ol-2*	*S. habrochaites* *S. lycopersicum*	Van der Beek et al., 1994
Late blight	*Phytophthora infestans*	*Ph-1*	*S. pimpinellifolium*	Pierce, 1971
		Ph-2	*S. pimpinellifolium*	Moreau et al., 1998
		Ph-3	*S. pimpinellifolium*	Chunwongse et al., 1998.

Fusarium crown and root rot	*Fusarium oxysporum* f.sp. *radicis lycopersici*	*Frl*	*S. peruvianum*	Berry and Oakes, 1987
Corky root	*Pyrenochaeta lycopersici*	*Pyl*	*S. peruvianum*	Laterrot, 1978
Viruses				
Tomato mosaic virus	Tomato mosaic virus (ToMV)	*Tm-1* *Tm-2* *Tm-2*^{2}	*S. hirsutum* *S. peruvianum* *S. peruvianum*	Pelham, 1966 Laterrot and Pecaut, 1969 Hall, 1980
Tomato spotted wilt virus	Tomato spotted wilt virus (TSWV)	*Sw-5*	*S. peruvianum*	Stevens et al., 1995
Tomato yellow leaf curl virus	Tomato yellow leaf curl virus (TYLCV)	*Tylc* *Ty-1* *Ty-2*	*S. pimpinellifolium* *S. chilense* *S. habrochaites*	Kasrawi, 1989 Zamir et al., 1994 Hanson et al., 2000
Tomato leaf curl virus	Tomato leaf curl virus (TLCV)	*Tlc*	*S. pimpinellifolium*	Barenjee and Kalloo, 1987
Alfalfa mosaic virus	Alfalfa mosaic virus (AMV)	*Am*	*L. hirsutum* f. *glabratum*	Parrella et al., 1998
Potato virus Y	Potato virus Y (PVY)	*pot-1*	*S. habrochaites*	Legnani et al., 1995
Bacteria				
Bacterial speck	*Pseudomonas syringae* pv. *tomato*	*Pto*	*S. pimpinellifolium*	Pitblado and MacNeill, 1983
Bacterial spot	*Xanthomonas campestris* pv. *vesicatoria*	*Bs-4*	*S. pennellii*	Ballvora et al., 2001
Nematodes				
Root-knot nematode	*Meloidogyne incognita, M. arenaria*	*Mi,Mi-1, Mi-3,Mi-9*	*S. peruvianum*	Smith, 1944
Potato cyst nematode	*Globodera restochiensis*	*Hero*	*S. pimpinellifolium*	Ellis and Maxon-Smith, 1971

5.5 Development of Cultivars Adapted to Abiotic Stresses

5.5.1 Salinity

Many areas where tomato is cultivated are affected by the problem of salinity. The cultivated tomato is classified as being “moderately sensitive” to salinity. It tolerates an EC (electric conductivity) of the saturated soil extract up to about 2.5 dS m^{-1}

without any yield reduction (Maas, 1986). Salinity affects different processes of plant life, mainly the percentage and speed of germination. Salinity also slows tomato shoot growth, and both stem and leaf dry weights are diminished in saline conditions. At low ECs, yield reduction is caused mainly by a reduction in the average fruit weight, while at high ECs the declining number of fruits explains the main portion of yield reduction.

Variability has been found for most of the above characteristics, and genotypes with a high expression of those characteristics have been identified within *S. lycopersicum* or in closely related wild species, such as *S. cheesmanniae, S. chilense, S. pennellii, S. peruvianum* and *S. pimpinellifolium* (Saranga et al., 1991; Foolad, 1997; for a review see Cuartero and Fernández-Muñoz, 1999). Heritabilities for most of the characteristics involved in salt tolerance that have been found in studies suggest that those characteristics can be improved by selection (Saranga et al., 1992). However, breeding for salinity has been slow due to various reasons, such as the difficulty of the evaluation of tolerance to salinity itself, the genetic control of most characters implicated, which is predominantly quantitative, and the impossibility of finding a single source of resistance which contains all characters of interest. Monogenic characters can be introgressed through backcrossing programs, but most of the characteristics involved in the tolerance to salinity have to be introgressed using recurrent selection programs. This makes the breeding process difficult and long.

Given the evidence that supports that tolerance to salinity is determined by multiple gene loci, the identification of QTLs associated with characters of interest and marker-assisted selection is another alternative that is being studied extensively. Several QTLs have been identified in intergeneric crosses (Bretó et al., 1996; Monforte et al., 1996), but some of the QTLs were shown to be dependent on the parentage of the cross (Monforte et al., 1997a) or on the saline or non-saline conditions, the QTLs being "constitutive" (those detected under both conditions), "response sensitive" (only detected under optimal conditions) or "response tolerant" (whose expression depends on the presence of salinity) (Monforte et al., 1997b; Foolad et al., 1999). QTLs associated with tolerance also vary with the stage of plant development. The QTLs associated with tolerance at germination (Foolad et al., 1997, 1998) and vegetative growth (Foolad and Chen, 1999; Foolad et al., 2001) differ (Foolad, 1999). Given that salinity is variable in time and space, MAS should take into account not only the constitutive and response tolerant, but also the response-sensitive QTLs. The different expression of a valuable QTL depending on the salinity level, the existence of epistatic interaction and pleiotropic effects (Monforte et al., 1999) complicate the use of MAS even more in trying to improve salt tolerance in tomato (Cuartero et al., 2006).

Genetic transformation has also been employed as a tool for increasing tolerance to salinity and has primarily used genes that code for transport proteins of ions and protons, such as the genes *AtNHX1, HAL1, BADH-1* (Arrillaga et al., 1998; Gisbert et al., 2000; Moghaieb et al., 2000; Rus et al., 2001; Zhang and Blumwald, 2001; Pineda, 2005). Overall, the results obtained suggest that the expression of individual genes in transgenic plants can increase salinity tolerance, at least to some extent.

Breeding of tomato cultivars tolerant to moderate salinity will probably occur after pyramiding in a single genotype several characteristics which alone could not confer a significant increase in tolerance. At present, no variety so far developed can be grown under saline conditions.

5.5.2 Extending the Thermal Range

Most tomato genotypes decrease their vegetative development at temperatures below 13ºC, with the fruit set being greatly affected (Picken, 1984). Poor winter fruit set is mainly due to a reduction in both pollen quality and quantity, since low temperatures (6 ºC) do not negatively affect ovule viability nor the early development of embryos. Two strategies have been employed to improve the set of the fruit at low temperatures: parthenocarpy, which is the formation of seedless fruits in the absence of functional pollination or other stimuli, and the production of fertile pollen at low temperatures. Regarding the first option, the genes *pat* (Soressi and Salamini, 1975), *pat-2* (Philouze and Miassoneuve, 1978) and *pat-3/pat-4* (Nuez et al., 1986) have been extensively studied. The *pat* gene has been recently mapped to the long arm of chromosome 3 (Beraldi et al., 2004) and many physiological studies have been conducted for *pat* (Mapelli et al., 1978; Mazzucato et al., 1999), *pat-2* (Fos and Nuez, 1997; Fos et al., 2000, 2003) and *pat-3/pat-4* genes (Fos et al., 2001). However, pleiotropic effects of these genes over other characteristics related to yield, quality and set of the fruit have been detected. This is the reason its use has not become effective. Regarding the quality of the pollen at low temperatures, a great variability for the availability of pollen production at low temperatures has been found in some wild species related to tomato, especially those which grow at high altitudes, such as *S. peruvianum* and *S. habrochaites*. However, the genetics of tolerance to low temperatures has not been well studied.

5.5.3 Long Shelf Life

The development of the "long shelf life" cultivars was carried out as a solution to the problem created by the loss of quality of tomatoes, normally collected at an immature state and therefore without the required gustative quality. This practice, which risks offering the consumer a product of lesser quality, is carried out because of the necessity of losing the minimum possible percentage of fruits from their harvesting to their arrival to the consumer.

The approaches used to extend the life of tomatoes have included the use of natural ripening mutants and antisense technology. Diverse mutants are known to affect the maturation process, both the synthesis of lycopene and the degradation of chlorophyll. Of these, the most used for the development of "long shelf life" cultivars have been the *rin* (*ripening inhibitor*) gene in a heterozygous state and the *nor* (*non-ripening*) gene. Prof. Kedar conducted a long breeding program in Israel which led to the release of the 'Daniella' cultivar which has a shelf life of about two months. In spite of the loss of quality associated with the use of these mutants, which have a delayed ripening of the fruit, the greater importance of the retailer over the

consumer in the producer-consumer chain has led to the great success of these mutants, thus changing the rules of the European production of the last years.

6 Current Goals of Breeding

6.1 Resistance to Insects in Tomato

The use of resistance to insects in tomato has been limited until now due mainly to the requirements for pest control in vegetable crops, where market standards are very strict. Another factor which has difficult the development of insect-resistant cultivars in general is that resistance can be difficult to quantify, making the selection process complicated and time consuming. The complex inheritance of the resistance is one more factor. However, considerable work has been conducted in tomato (Farrar and Kennedy, 1991).

In general, the mechanisms of resistance in tomato can be divided into two groups: a) those which are associated with foliar trichomes (glandular or non-glandular) and with the substances secreted, and b) those associated with the leaf lamella, fruit, or plant growth habit.

Trichomes are uni- o pluricellular structures which cover the surface of stem and leaves. They are considered as differentiate epidermal cells. Classification of trichomes is based on its functionality and morphology. Regarding its functionality, trichomes can be glandular or non glandular, holding the glandular type cells that secrete different substances. Classification of trichomes in tomato was made by Luckwill (1943), who distinguished seven types of trichomes, named from I to VII. Channarayappa et al., (1992) reviewed the classification made by Luckwill and added one more type. The most studied and effective resistance is that conferred by glandular trichomes, which are those of I, IV, VI and VII types, being the main those of IV and VI types. This resistance is based on two non-excluyent mechanisms: antixenosis or the mechanis that limit the possibility of contact between the arthropod and the plant (repellence is an example of this mechanism), and the antibiosis or the mechanism that causes the mortality or diminishes the growth rate.

Resistance to insects has been found in almost all the wild relatives of tomato, but mainly in *S. habrochaites* and *S. pennellii*. In *S. habrochaites* resistance is conferred by trichomes IV and VI, while in *S. pennellii* the resistance is conferred by trichomes of type IV. Trichomes of type IV have also been identified in *S. pimpinellifolium* (Fernández Muñoz et al., 2003). Resistance up to 16 pest species has been reported in accessions of S. *habrochaites*. One accession, PI 134417 has been intensively studied, being resistant to some 12 arthropod pests of cultivated tomato. Another promising source of arthropod resistance is *S. pennellii*. Resistance to nine arthropod pests has been reported in this species, with one accession, LA 716, being resistant to eight of these pests.

One of the most thoroughly studied arthropod-resistance factors in tomato or any crops is the 2-tridecanone which is found in the tips of Type VI glandular trichomes (of *S. habrochaites* var. *glabratum* (Williams et al., 1980). This compound is highly toxic to many pests such as *Tetranychus urticae* (Snyder et al., 1993), *Bemisia tabaci*

(Channarayappa et al., 1992) and *Trialeurodes vaporariorum* (Maliepaard et al., 1995) and act as a repellent to others. The genetic control of 2-tridecanone is complex. Inheritance of high levels of 2-tridecanone is controlled by at least three independently assorting recessive genes (Fery and Kennedy 1983, 1987; Fery et al., 1984). Among 3 to 5 QTLs have been implicated in the synthesis of 2-tridecanone in crosses between *S. lycopersicum* and *S. habrochaites* (Nienhuis et al., 1987; Zamir et al., 1984). There are several factors that could potentially complicate the use of 2-tridecanone-based resistance in pest management. It has been demonstrated that 2-tridecanone induces detoxifying enzymes in some insects such as *Heliothis* spp. recovering from the initial intoxication. It has also adverse effects to natural enemies of *H. zea* and other pests. These facts need to be considered in any plant breeding program where the goal is the development of cultivars with 2-tridecanone-based host plant resistance.

Other group of compounds related to resistance is the sesquiterpenes found in *S habrochaites* var. *hirsutum.* Genes that synthesize different sesquiterpenes have been cloned employing near isogenic lines NILs (van der Hoeven et al., 2000).

Acylsugars which are present in the exudates of type IV trichomes are the responsible of the resistance in *S. pennellii.* One of the most intensively studied accessions of this species is LA 716. This accession has shown a very high level of resistance to the whitefly *Bemisia tabaci/B argentifolii* complex, as well as to aphids (*Macrosiphum euphorbiae, Myzus persicae*), mites and Lepidoptera pests, including the South American tomato pinworm *Tuta absoluta* (Resende et al., 2006). At least 5 QTLs are involved in the inheritance of acylsugars in accession LA 716 (Blauth et al., 1998). A marker-assisted selection program was conducted to transfer the ability to accumulate acylsugars from *S. pennellii* LA 716 to cultivated tomato (Lawson et al., 1997). The results indicated that it is likely that another not identified regions of the genome could be implicated in the ability to accumulate acylsugars. More accurate studies to locate all the regions implicated are necessary before conduct a marker assisted program. Moderate values of heritability, $h^2_b = 0.476$ have been found by Resende et al., (2002).

The whitefly *Bemisia argentifolii* is one of the key pests of tomato in many parts of the world. Screening to find sources of resistance has been conducted. Accessions LA 716, LA 1340 and LA 2560 of *S. pennellii* and LA 1777 and LA1353 of *S habrochaites* f. *typicum* were selected as the most resistant (Muigai et al., 2003). Both behavioural and toxic effects were acting as mechanisms of resistance in these experiments. Regarding the components of exudates of glandular trichomes of *L. habrochaites*, the methyl ketones 2-tridecanone, 2-undecanone and 2-pentadecanone were studied. It was determined that 2-tridecanone has low levels of repellent and residual toxicity activity, that 2-undecanone has higher levels of repellent and fumigant activity; and that ginger oil (compound in part of sesquiterpene hydrocarbons) had high levels of repellent and residual toxicity activity. These studies suggest that multi-factor resistance exists in wild tomato germplasm. Thus, the combination of several factors seems to be the best strategy to difficult the overcome of the resistance by *B. argentifolii* (Muigai et al., 2002). Attempts to locate the whitefly resistance loci to *B. argentifolii* in accession LA177 have been

conducted by Momotaz et al., (2005) using the recombinant inbred lines developed from *S. habrochaites* LA 1777 by Monforte and Tanksley (2000a).

The two-spotted spider mite (*Tetranychus urticae*) has also been extensively studied as it is one of the most devastating pests of tomato. Although some levels of resistance have been found in *S. lycopersicum* (Knaap et al., 2003), higher levels of resistance have been found in the wild relatives, mainly in *S. habrochaites*. Extracts from several accessions of *S. habrochaites* exhibited high lethal, antibiotic and repellent activities. Lethality of extracts was associated with the presence of high concentrations of 2-tridecanone, whereas repellence of extracts was mainly associated with trans-caryophyllene (Antonious and Snyder, 2006). Both repellent and antibiotic activities were transferred to interspecific tomato hybrids (Snyder et al., 2005). Resistance to *Tetranychus urticae* has also been detected in *S. pimpinellifolium* (Fernández-Muñoz et al., 2000). The genetic of the resistance has been studied by Fernández-Muñoz et al. (2003) in the accession TO-937. Resistance was found to be controlled by a single dominant major locus, but modulated by unknown minor loci. The presence of glandular trichomes type IV was governed by two dominant unlinked loci.

Inside the b) group, those associated with the leaf lamella, fruit, or plant growth habit, there are several non-trichome-based factors which have been implicated in host plant resistance to arthropods. Perhaps the best known of these factors is the glycoalcaloid α-tomatine present in all the wild relatives of tomato except in *S. pennellii*. This type of resistance has been much less studied that those conferred by trichomes.

In spite of the considerable work conducted in tomato no cultivars with resistance to insects are currently commercialized.

6.2 Resistance to Diseases

Once dominant genes of resistance to a certain disease have been identified, their introgression in commercial hybrids has traditionally solved the problem. In some cases these genes have remained useful for many years. This is the case of the *Tm2*^{2}, *Sm* and *Mi* genes (Scott, 2005). On occasion, the appearance of new races of the pathogen has forced the search for new genes, as in the case of the *I, I-2* and *I-3, Ph-1, Ph-2* and *Ph-3* genes, or the long series of *Cf* genes. In this last case, the joint use of more than one gene in hybrids (*Cf-2* along with *Cf-5* is the most common combination) confers a more durable resistance. However, polygenic resistance has been less used in tomato due to the partial resistance it confers and to the difficulty of its management in breeding programs. The current problems with breeding for resistance to diseases are different when taking into account fungi, bacteria or viruses, and so we will deal with each separately.

6.2.1 Bacteria

The bacterial diseases that cause the most significant economic losses in tomato are bacterial leaf spot, which is produced by four different species of *Xanthomonas*: *X. euvesicatoria, X. vesicatoria, X. perforans* and *X. gardneri* (Jones et al., 2004),

bacterial wilt, which is caused by *Ralstonia solanacearum,* bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* and bacterial speck caused by *Pseudomonas syringae* pv. *tomato*. Genetic resistance to *Pseudomonas syringae* is conferred by the *Pto* gene (Pitblado and MacNeill, 1983), and resistant cultivars have been developed. However, the general situation at present is that there is a lack of dominant genes of resistance against bacteria.

Breeding for bacterial disease resistance in tomato has not resulted in many resistant cultivars because most resistances are multigenic, do not provide complete protection against the pathogen under certain environmental conditions and unfavourable linkages sometimes exist (Scott, 1996). This is the case of bacterial wilt produced by *Ralstonia solanacearum*. This pathogen harbours a very high genetic diversity and infects a wide range of plant families. Resistance is complex and strongly influenced by environmental conditions such as soil temperature, pH, and moisture, and this makes the selection of resistant plants quite complicated. Additionally, the fact that the most commonly used resistance sources come from *S. lycopersium var. cerasiforme* and *S. pimpinellifolium* has made the development of big-fruited resistant cultivars even more difficult because of the existence of a linkage of the resistance to the small size of these species. In light of these circumstances, extensive studies of this pathogen have been conducted worldwide with the aim of gaining a better understanding of the most adaptable germplasm and of finding the best sources of resistance for any given region of the world. Thirty-one bacterial wilt-resistant genotypes derived from at least 14 resistant sources have been tested in 11 countries (Wang et al., 1998; Scott et al., 2005). Seven genotypes with resistance levels of approximately 90% have been identified. The sources of resistance come from Hawaii, the Philippines and North Carolina (although these sources had Hawaiian-like phenotypes). Although the existence of a dominant gene for which molecular markers have been identified has been reported (Wang et al., 2000), contradicting results have been found in different assays (Scott et al., 2005) due to the large effect of environmental conditions on resistance expression and the unclear nature of the genetic control of resistance to this pathogen. One quite positive result of this international project is that the most resistant genotypes can now be used in the development of resistant varieties both with a broad adaptation as well as for more specific regions.

Bacterial canker of tomato, caused by *Clavibacter michiganensis* subsp. *michiganensis* is a devastating disease of tomato. The only resistance that has been found is a partial resistance in the green-fruited species *Solanum habrochaites*, accession LA407 (Francis et al., 2001) and *S. peruvianum* accession LA2157 (Sandbrink et al., 1995; Van Heusden et al., 1999). These sources of resistance appear to be similar (Francis et al., 2001). Two QTLs from *Solanum habrochaites,* Rcm 2.0 and Rcm 5.1, which control resistance to *Clavibacter michiganensis* subsp. *michiganensis,* were identified and introgressed into a tomato cultivar using inbred backcross breeding (Kabelka et al., 2002). The mechanism of action of these two QTLs seems to be different (Coaker et al., 2002; Kabelka et al., 2002). The genes have been fine-mapped on chromosome 2 (Rcm 2.0) and chromosome 5 (Rcm 5.1) and it has been demonstrated that they interact epistatically (Coaker and Francis, 2004). The availability of molecular markers closely linked to these QTLs will

facilitate their introgression in tomato and the development of resistant cultivars. Additionally, the construction of an *S. habrochaites* LA 407 large-insert transformation competent artificial chromosome (TAC) library (Qu et al., 2003) will probably permit chromosome walking towards the genes underlying Rcm 2.0 and Rcm 5.1 and the possibility of their cloning. Apart from this resistance, four QTLs have also been identified in *S. neorickii*, which confirms the quantitative nature of the resistance to *Clavibacter michiganensis* subsp. *michiganensis* in tomato (Ganal et al., 1999).

Bacterial spot of tomato is caused by *Xanthomonas campestris* pv. *vesicatoria*. The strains of this bacterium have been divided into three groups, according to host-range specificity. The XcvT group includes strains that are pathogenic on tomato, the XcvP group contains strains pathogenic on pepper, and the XcvTP group includes strains that are pathogenic on both species (Minsavage et al., 1990). Three races, T1, T2 and T3, have been identified among the strains belonging to XcvT and XcvTP on the basis of the reaction they elicit from three tomato cultivars (Jones et al., 1998; Stall, 1995). Some resistance sources have been identified (Scott et al., 1996). However, the complex inheritance and problems in obtaining resistance in determinate genotypes with large fruit have made the development and release of resistant cultivars difficult. A dominant gene elicits a hypersensitive response (HR) in leaves of *S. pennellii* LA 716. A single dominant gene controls the resistance in this accession (Astua-Monge et al., 2000a and b). In spite of the feasibility of the use of this resistance, it has not been used extensively as it does not confer resistance to other species of *Xanthomonas*. The problem with this pathogen remains unsolved.

A significant advance in the breeding for resistance to bacterial diseases could be obtained if molecular markers to all the known genes of resistance were available. Epistatic relations providing resistance to pathogens present in a certain area of production could be identified by means of inoculation tests of different lines that are carriers of different combinations of genes of resistance with several races of the pathogen. Once the necessary combination of genes has been identified, they can be incorporated in locally adapted cultivars using the molecular markers previously identified. Cases of epistatic relationships between genes of resistance to bacteria have already been identified, either between different races of a pathogen or even between different pathogens (Scott et al., 2005).

When effective resistance is not known, as in the case of the bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria,* alternative methods can be employed. In such cases, systemic acquired resistance (SAR) compounds have become a popular alternative to conventional bactericides. One that has shown promise for the control of bacterial spot is acibenzolar-S-methyl (ASM). The application of bacteriophages that are specific to the bacterial spot pathogen has also given positive results (Jones et al. 2005).

6.2.2 Fungi

The great success obtained in the development of cultivars resistant to fungi has been due to the availability of dominant genes of resistance (see Table 7). One of the remaining unsolved problems is the early blight produced by *Alternaria solani*. It is

one of the few cases in tomato in which horizontal resistance has been employed because of a lack of monogenic resistances (Foolad et al., 2000). Several sources of resistance have been identified in *S. habrochaites* and *S. pimpinellifolium* and they have been introgressed in tomato (Gardner and Shoemaker 1999; Foolad et al., 2005). However, the level of resistance of these hybrids is insufficient. The resistance to early blight has been characterized as a complex quantitative character controlled by additive and nonadditive interactions of multiple genes and is highly influenced by environmental effects (Nash and Gardner, 1988). In addition, the expression of the resistance is associated with the plant's physiological maturity and load of fruits. Due to these difficulties, advancement in the development of resistant cultivars has been slow and has had limited success. In cases such as this, the development of molecular markers and high-density genetic maps can facilitate the development of resistant cultivars. Some QTLs have been identified and mapped in interspecific crosses between *S. lycopersicum* and *S habrochaites* and between *S. lycopersicum* and *S. pimpinellifolium*. The most important QTLs identified in *S habrochaites* are located on chromosomes 5, 8, 9, 10, 11 and 12 (Foolad et al., 2002), whereas the most promising QTLs in *S. pimpinellifolium* have been located on chromosomes 1, 3, 4, 5 and 6. This suggests that the resistant accessions of *S habrochaites* and *S. pimpinellifolium* could have different genes of resistance to early blight, thus presenting the opportunity of pyramiding the QTLs of these two sources of resistance and introgressing them into elite cultivars (Foolad et al., 2005).

6.2.3 Viruses

As mentioned in the previous section, some viral diseases have been successfully controlled by resistance genes. This is the case of Tomato mosaic virus and Tomato spotted wilt virus. However, there are several viruses for which either no genes of resistance are known or the available genes of resistance are isolate-specific. Examples of this situation are Pepino mosaic virus, the complex of geminiviruses which infect tomato and several Criniviruses.

Pepino mosaic virus is an emerging disease in greenhouse tomato production. It was first detected in Peru in 1980 in pepino (*Solanum muricatum*) (Jones et al., 1980). Much later, in 1999, it was detected in greenhouse tomato crops in the Netherlands and the United Kingdom (van der Vlught et al., 2000). It has spread throughout Europe (Verhoeven et al., 2003) and North (French et al., 2001) and South America (Ling and Soler et al., 2002; Ling and Carpenter, 2005). Isolates from different geographic regions share very high genome sequence similarity, between 99 and 100% (Mumford and Metcalfe, 2001; López et al., 2005). The virus is easily spread by mechanical means. It has recently been demonstrated that PepMV is present in the seeds, concentrated in the seed coat, and not in the embryo. However, the virus is not easily transmitted to germinating tomato seedlings from virus-infected tomato seeds (Lingh and Carpenter, 2005). Pepino mosaic virus has been associated with the collapse of tomato plants detected in greenhouse-cultivated tomato in Almeria, Spain (Soler et al., 2005). The presence of PepMV in all collapsed plants suggests a relationship between this Potexvirus and tomato collapse. Many accessions of wild tomato relatives have been screened in searches for

resistance that have had little success. Only partial resistance has been found in some accessions of *S. chilense*, mainly in accession LA470. The species *Solanum pseudocapsicum* and *S. ochrantum* exhibited a high level of resistance, as the virus could not be detected by ELISA assay (Soler et al., 2005). However, the strong incompatibility barriers that exist between these species and the cultivated tomato make their exploitation in plant breeding problematic.

So far, 34 recognised and 18 tentative species of begomoviruses (belonging to the Geminiviridae family) have been found to naturally infect tomato (Varma and Malathi, 2003). The most devastating begomoviruses affecting tomato are those with the generic names 'tomato leaf curl virus' and 'tomato yellow leaf curl virus'. These viruses are widely distributed throughout Africa, the Americas, Asia, Australia and parts of Europe. Nine begomovirus species and five more proposed species associated with tomato yellow leaf curl disease (TYLCD) have been identified (Fauquet and Stanley, 2005). The worldwide spread of the B biotype of *Bemisia tabaci*, the vector of the virus, has facilitated the spread of whitefly-transmitted geminiviruses. Breeding for resistance or tolerance to the virus is the best way to control the disease, given the impossibility of effectively controlling the vector. Resistance has been found in tomato wild relatives (reviewed in Laterrot 1992; Picó et al., 1996, 2000; Pilowsky and Cohen, 2000). However, the progress in breeding resistant tomatoes has been very slow due to the lack of accurate inoculation methods and plant resistance-level assessment (Picó et al., 1998; Lapidot et al., 2000). In fact, several factors, such as plant age at the time of infection, inoculation pressure and growth conditions, all have a major effect on the severity of symptoms. A panel of differentiated hosts has been developed by Lapidot et al. (2001) to facilitate the assessment of resistance levels. The first resistant commercial hybrid, TY-20, carrying resistance from *S. peruvianum* was developed by Pilowsky and Cohen (1990). Later, breeding lines with high levels of resistance derived from *S. peruvianum* (Lapidot et al., 1997; Friedmann et al., 1998), *S. chilense* (Zamir et al., 1994; Scott et al., 1996; Picó et al., 1999; Pérez de Castro et al., 2005), *S. pimpinellifolium, S. peruvianum* (Vidavsky et al., 1998) and *S. habrochaites* (Vidavsky and Czosnek, 1998; Hanson et al., 2000) were developed by different research teams (reviewed in Lapidot and Friedmann, 2002). Different genetic controls of resistance to TYLCV have been described. At least three interacting genes are responsible for the resistance in line TY172 developed from *S. peruvianum* (Friedmann et al., 1998). The resistance in *S. habrochaites* appears to be controlled by two to three additive recessive genes (Vidavsky and Czosnek, 1998) and by two genes acting epistatically (Banerjee and Kalloo, 1987) in the sources used by the AVRDC (Asian Vegetable Research and Development Center). Later, these researchers developed the line H-24, which carries the gene *Ty-2* derived from *S. habrochaites* (Hanson et al., 2000, 2006). Different genetic controls have been found in s. pimpinellifolium, ranging from a simple dominant gene (Kasrawi, 1989) to quantitative and partially recessive or monogenic incompletely dominant (Pérez de Castro et al., 2007a). Resistance in *S. chilense* LA1969 is controlled by one major gene named *Ty-1* and at least two more modifier genes (Zamir et al., 1994). The resistance most commonly used in the construction of hybrids is the *Ty-1* gene. Although a great number of resistant breeding lines exist, and commercial hybrids

have been released and cultivated in areas with high levels of infection, a major adaptability of the materials to specific growing areas and a more varietal options is still needed.

Two viruses belonging to the genus *Crinivirus* (family *Closteroviridae*), Tomato chlorosis virus, ToCV (Wisler et al., 1998), and Tomato infectious chlorosis virus (TICV) (Dufus et al., 1996) appeared recently in Florida and California, respectively. They have since spread to the main growing areas of tomato. No genetic resistance to these viruses has yet been found.

Finally, interactions of resistance genes with other disease responses have been detected in materials carrying several genes of resistance. Such an interaction occurred with resistance to Tomato mottle virus (ToMoV). The reaction of the plants to the infection with ToMoV was different depending on whether they carried Tomato mosaic virus (ToMV) resistance at the *Tm-2/Tm-2*^{2} locus (Griffiths, 1998). This is another type of interaction that should be considered when breeding for resistance (Scott, 2005).

6.3 Breeding for Quality

Breeding for tomato quality has followed different objectives and methodologies according to the type of consumption: fresh or processed.

6.3.1 The Concept of Quality in Tomato for Processing

Tomatoes for processing have to combine certain quality requisites, both external and internal. Regarding the external aspect, they have to be homogeneous in size and shape. This is essential in conducting a good mechanical harvest and for the production of whole peeled tomato. Other essential requisites are a uniform, intense red colouring, lack of cracking, small pedicel scar and flexible skin which permits easy peeling. As far as the internal characteristics, it is important for the outer and radial pericarp walls to be heavy and firm in order to reduce losses during harvesting and transport to a minimum; the central pericarp column must be firm and thick but not suberified; lack of puffiness in the locules and with an optimum number of three. The yield in the processing of certain manufactured types depends on a series of internal quality parameters, for instance colour, which must be an intense red, licopene being the carotenoid that contributes the greatest red tonality, whereas others contribute different tones, such as β-carotene, which contributes orange or yellowish tones. The soluble solid content is the parameter that most influences the yield in the production of concentrated tomato (Yousef and Juvik, 2001). Current cultivars should range between 4 and 6ºBrix. The viscosity depends on the quantity of soluble pectins and directly affects the consistency of different products, such as juice, ketchup and sauces. The pH is directly related to the conservation of the final product, and must be below 4.4. Finally, the flavour and the scent are two characteristics whose measurement is very complex, with the influence of the sugars:acids ratio being very important. In addition, a large number of volatile components affect the aroma. The industrial processes can be optimized to maintain both the flavour and the aroma as much as possible. This serves to highlight the fact

that, depending on the type of end product, varieties must have different quality characteristics. Thus, tomatoes destined for whole peel and diced product have different quality parameters than those destined for paste.

Flavour breeding programs started more than 40 years ago in tomato for processing and have been based on the variability found in landraces of *S. lycopersicum* and the *cerasiforme* variety, as well as the wild relatives *S. chmielewskii* and *S. cheesmannii*, for the most part. The general scheme of these breeding programs has been the backcross method, which alternates selfing generations and selection (Hewitt et al., 1987). Additionally, the evaluation by genotype of the clones of several plants and the regrouping of positive alleles by means of intercrossing has increased the success of selection in these breeding programs (Gragera, 2006). Tomato processing cultivars that suitably fulfil most of the indispensable requirements as far as morphology and external aspect go have been obtained through this process (Gardner 1993 a and b, 2006 a and b). Therefore, the most recent projects are mainly focused on colour and lycopene content, soluble solid content, juice consistency, pericarp firmness, aroma and flavour. All these characteristics are polygenic, often negatively correlated among themselves and highly influenced by environmental factors.

One approach to improving the colour and lycopene content has been the use of genes that affect the colour and biosynthesis of carotenoids. The most important genes are *at, B* and its allele og^c, *Del, dps*, *gf*, *gh*, *Gr*, *hp-1*, *hp-2*, *Ip* and its allele *dg*, MO_B, *ry*, *t*, *r*, *sucr,* etc. (Wann et al., 1985; Chalukova and Manueyan, 1991; Chetelat et al., 1993, 1995; Kabelka et al., 2004). Many of these genes have been used in improving fruit quality, sometimes by employing marker-assisted selection. RAPD and AFLP (Zhang and Stommel, 2000) and SCAR and CAPS linked to the *B* and *MoB* genes have been identified, thus facilitating their introgression in breeding lines. Thus, many tomato cultivars for processing have introgressed genes for colour and high lycopene content.

More recently, the development of high-density genetic maps and the availability of DNA molecular markers have permitted the localization and management through marker-assisted selection of QTLs associated to various characters related to quality. In this way, the use of a pedigree method for the management of major genes has been substituted by a population-based approach for the manipulation of quantitative traits. The advanced backcrossing method, which allows the identification of beneficial alleles in unadapted germplasm and simultaneous transfer into elite cultivars, has permitted the identification of QTLs related to different aspects of quality in *S. pimpinellifolium, S. pennellii, S. peruvianum* and *S. habrocaites*. As a result, QTLs related to soluble solids, juice consistency and pericarp firmness, viscosity, colour, flavour and aroma have been identified and mapped (Paterson et al., 1990, 1991; Garvey and Hewitt, 1992; Eshed and Zamir, 1994; Goldman et al., 1995; Grandillo and Tanksley, 1996; Tanksley y Nelson, 1996; Tanksley et al., 1996; Fulton et al., 1997, 2000, 2002a; Bernacchi et al., 1998a, b and c; Bucheli et al., 1999; Chen et al., 1999; Egashira et al., 1999; Monforte and Tanksley, 2000b; Frary et al., 2004; Kabelka *et al*., 2004; Yates et al., 2004). Although breeding programs based on exclusively phenotypic selection are still conducted, the selection of QTLs based on marker-assisted selection is rapidly being adopted. A selection program has

been conducted to transfer QTLs for organoleptic quality from a cherry tomato characterized by its good flavour into the genetic backgrounds of several large fruits (Saliba Colombani et al., 2001; Causse et al., 2001 and 2002; Lecompte et al., 2004). The largest cluster of QTLs was located in the distal region of chromosome 2 (a region of 50cM). A marker-assisted selection scheme was performed in order to transfer five regions of the cherry tomato line into the three lines. A marker-assisted backcross program was effective and quickly accumulated up to five QTLs in a single genotype. Other breeding programs have been conducted by Baxter et al. (2005a) and Yousef and Juvik (2001) using QTLs for soluble solids content and by Francis et al. (2003) for fruit colour and juice viscosity, all with positive results. Francis et al. (2003) recommends the application of MAS during the first generations of selection, whereas phenotypic evaluation in the more advanced phases of breeding programs is still irreplaceable.

6.3.2 The Concept of Quality in Tomato for Fresh Consumption

Contrary to what has occurred with breeding for quality in tomato for processing, where quality has been a high-priority objective from the beginning, in tomato for fresh consumption, tomato breeders have mainly emphasized yield, fruit size and appearance (lack of defects and an attractive colour), disease resistance and, more recently, fruit firmness and an adequate shelf life. Consequently, internal tomato quality has decreased markedly, and consumers demand fruits of higher quality.

In tomato for fresh consumption, quality is a combination of external (size, colour, shape) and internal factors. Internal quality can be divided into organoleptic and nutritional qualities. Organoleptic quality is related to flavour (sweetness, acidity, etc.), olfactory (aroma, scent) and tactile aspects (firmness, texture, etc.). The flavour is determined mainly by the content of organic acids and reducing sugars. Reducing sugars represent approximately 50% of the dry weight (dw), and organic acids, mainly citric and malic acids, represent more than 10% dw (Chamarro, 2003). The aroma is determined by volatile compounds. Of the 400 volatile compounds that have been identified in tomato, approximately 30 are responsible for the aroma of fresh tomato. When tomato is fresh–consumed, all these components are perceived directly by the consumer.

In breeding for quality of tomato for fresh consumption, the most important sources of variation have been the wild relatives of tomato, the landraces, which have conserved their high quality because they have never been subjected to breeding processes or to tomato varieties for processing. Given the slowness in obtaining positive results when selection is made for high total soluble solids content, one strategy suggested by some authors is to substitute the measurement of ºBrix, pH and titratable acidity for precise chemical determinations of each of the non-volatile components related to flavour (Roselló et al., 2002; Roselló and Nuez, 2006). The development of analytical techniques that permit a quick and precise quantification of each quality component is necessary in order to test the high number of plants required in breeding programs. Thus, high performance liquid chromatography (HPLC) (Baldwin et al., 1991; Berg and Canessa, 1998) and capillary zone electrophoresis (CZE) (Galiana-Balaguer et al., 2001; Roselló et al.,

2002) are being optimised and used today to determine individual organic acids (mainly citric, malic, oxalic and glutamic) and sugars (mainly glucose, fructose and sucrose). The individual quantification of each component can facilitate the development of breeding lines with a specific gustative profile.

The utilization of genes that affect colour and the biosynthesis of carotenoids as well as the identification of QTLs related to quality used in breeding for quality of tomato for processing are also being employed in the breeding of tomato for fresh consumption.

6.3.3 Nutritional value

Special attention to the nutritional quality of foods is required at present given the increasing interest in functional foods. The nutritive value of tomato is not very high (Table 8). However, its high level of consumption makes it one of the main sources of vitamins and minerals in many countries.

Table 8. Vitamin content in tomato fruit (from Adalid et al., 2004).

Vitamin	Content (μg/100 g fw)
Ascorbic acid (vitamin C)	25,000 – 30,000
β-carotene (provitamin A)	900 – 1,271 iu*
Tocopherol (vitamin E)	40 – 1,200
Nicotinic acid (niacin)	500 – 700
Pantothenic acid (vitamin B3)	50 – 750
Pyridoxine (vitamin B6)	80 – 110
Thiamine (vitamin B1)	50 – 60
Riboflavin (vitamin B2)	20 – 50
Folic acid	6.4 – 20
Biotine	1.2 – 4

* iu: internacional units = 0.6 μg β-carotene

Nutritional quality of tomato is mainly determined by its lycopene and vitamin C and E contents, although most research has been directed towards increasing the level of lycopene. Between 90-95% of the carotenoids present in ripened tomatoes are carotenes. Lycopene is the most abundant carotene in the red tomato fruit, accounting for up to 90% of the total. Lycopene has important dietetic properties since it reduces the risk of several types of cancer and heart attacks (Dorgan et al., 1998; Clinton, 2005). β-carotene is a provitamin A carotenoid and its deficiency can cause xerophtalmia, blindness and premature death. Carotenoid variations from more than one- to three-fold (β-carotene varying from 1.15 to 3.7 mg/kg fw and the carotenoid total varying from 18.5 to 60.7 mg/kg fw) have been reported in several tomato cultivars (Abushita et al., 1997). In tomato processing cultivars, variations in lycopene content ranged from 1,000 to 2,000 mg/kg dw. In *S. pimpinellifolium* the average values for lycopene content were as much as five times higher than those found in the cultivated tomato (Fernández-Ruiz et al., 2002), making these accessions a promising source of variability for increasing the lycopene content in tomato breeding programs.

Transgenics has been used extensively in breeding tomato for both organoleptic and nutritional quality, with positive results in many cases. This aspect will be reviewed in Section 8.

7 Breeding Methods and Techniques

Several breeding methods and techniques are required for the development of a new commercial variety. For example, in order to obtain a commercial hybrid, the following methods and techniques can be applied: crosses between different lines or hybrids with complementary characteristics in order to generate a segregant population, the pedigree method to develop inbred lines from this segregating population with the required characteristics, the backcross method to introgress a specific characteristic in a line as well as others. In the development of inbred lines, embryo rescue and in vitro culture can be used to accelerate the process. Alternatively, anther culture can be employed in order to obtain inbred lines in a shorter period of time. Test experiments that check the resistance to certain diseases have to be performed to select resistant plants. Marker-assisted selection can also be used for this purpose if molecular markers are known for a particular gene. Finally, many crosses have to be conducted in order to find the most heterotic combinations. The manipulation of quantitative traits is more complicated. In this case, recurrent selection has been used to construct populations with a higher level of expression of a specific character. Inbred lines can be obtained from this population by the pedigree method. We will consider in more detail some of these methods.

Interspecific hybridization has been used extensively in tomato due to its narrow genetic basis. Embryo rescue has been used to produce interspecific hybrids due to the sexual incompatibility that exists between tomato and the wild *Solanum peruvianum* (Smith, 1944; Alexander, 1963; Thomas and Pratt, 1981; Poysa, 1990; Cap et al., 1991; Chen and Taiji, 1996: Picó et al., 2002). Some accessions of *S. peruvianum* have been shown to be more compatible with *S. lycopersicum* (Rick, 1983; Ayuso et al., 1987) and can be used as a genetic bridge in transferring characteristics of interest from other accessions of *S. peruvianum* to *S. lycopersicum*. Backcrosses to *S. chilense* obtained from interspecific hybrids between *S. lycopersicum* and *S. chilense* have also been employed as a bridge for the introgression of genes of interest from incompatible accessions of *S. peruvianum* into *S. lycopersicum* (Picó et al., 2000). *S. lycopersicum* is not as incompatible with *S. chilense* as with *S. peruvianum*. In this case, the use of the pollen mixture method made obtaining interspecific hybrids possible. Other more distant species, such as *S. lycopersicoides,* which possess resistance or tolerance to several diseases affecting tomato as well as to chilling (Chetelat et al., 1997), have also been crossed to *S. lycopersicum* (Rick, 1951; Gradziel and Robinson, 1989; Chetelat et al., 1989), but the introgression of characteristics of interest was not possible due to the male sterility of the hybrids and their unilateral incompatibility when used as the pistillate parent. More recently, Chetelat et al. (1997) obtained a partially male-fertile F1 hybrid by sexual hybridization, which enabled the obtaining of BC1 plants. Monosomic alien addition lines (Chetelat et al., 1998) and a group of backcross-inbred

lines (Chetelat and Meglic, 2000) have also been obtained. Homeologous pairing and recombination have been studied in *Solanum lycopersicoides* monosomic addition and substitution lines of tomato (Ji and Chetelat, 2003). A genetic map of tomato based on BC1 *Lycopersicon esculentum* x *Solanum lycopersicoides* has been constructed showing overall synteny and suppressed recombination between these homeologous genomes (Chetelat et al., 2000). All of these studies open the possibility of using the genes of interest of *S. lycopersicoides* in tomato breeding. Hybrids with *Solanum sitiens* have also been obtained indirectly using sesquidiploid *L. esculentum* x *S. lycopersicoides* as a bridge (De Verna et al., 1990; Pertuzé et al., 2002). The chromosomal constitution and pairing of these hybrids has been studied by Ji et al. (2004).

The method used for introgressing characters controlled by a single major gene, both from wild species and from a different tomato cultivar, has generally been the backcross. This is the case of the R genes of resistance, whose control is usually monogenic. More recently, backcross has also been used for the development of Substitution Lines, Advanced Backcross and Backcross Inbred Lines. These populations signify advantages for the mapping and introduction of quantitative trait loci (QTLs) from exotic germplasm, including those related to yield and fruit quality or resistance to diseases (Bernacchi et al*.,* 1998a; Tanksley and Nelson, 1996; Tanksley et al*.,* 1996; Frary et al*.,* 2004).

Backcross combined with selfing generations has been employed in introgressing multigenic characters such as tolerance to salinity or other abiotic stresses. However, the level of expression of the specific character is not usually as high in the developed varieties as in the donor source due to the complexity of the genetic control. Thus, success in the development of cultivars tolerant to salinity has not been outstanding.

Recurrent selection has been employed in the development of many cultivars for processing but not generally with hybrids (Grajera et al., 2002). Recurrent selection has also been employed in the development of populations with enhanced resistance to pathogens when monogenic resistance is not available (Laterrot 1995).

Haploid selection has not been widely used in tomato breeding. However, attempts have been made to breed tomato for tolerance to low and high temperatures and for earliness. Zamir et al. (1982) demonstrated that the gametophytic selection for low temperature tolerance of *S. habrochaites* pollen is determined, at least in part, by genes expressed in the haploid pollen. Domínguez et al. (2005) were successful with one generation of gametophytic selection when it was applied in segregating populations derived from a *Solanum lycopersicum* x *S. pennellii* hybrid. However, the second cycle of selection showed no improvement. Alvarez et al. (1994) found that heat treatment generally affected pollen tube growth and mainly pollen germination, which suggests the possibility of using gametophytic selection for the improvement of tolerance against heat stress. Crispi and Pierce (1992) demonstrated the unsuccessfulness of this method in modifying the growth period.

Since the first studies that demonstrated the existence of heterosis in many characters related to yield, the development of hybrid varieties has been used extensively in the breeding of tomato for fresh consumption. Single seed descent, alternating test to know the general combining ability (crosses with testers with a

wide genetic basis) has been the most used method in the development of inbred lines parental of hybrids. These lines have been improved by introducing genes of resistance to different pathogens through backcross programs. Embryo rescue is also used to accelerate the development of inbred lines in this process. Embryos are rescued from immature fruits and cultured in vitro, making the complete ripening of the fruit unnecessary and shortening the generational period by one or two months. The single seed descent method has also been employed for the development of processing cultivars from populations, segregating for disease resistance, jointless character, and fruit characteristics, such as size, firmness and total solids (Giordano et al., 1997).

Doubled haploid technology is a powerful alternative to classic plant breeding strategies, mostly due to the significant time and resource savings that can be achieved through its application. At present, doubled haploids have been obtained in many crops of agricultural interest (see http://www.scri.sari.ac.uk/assoc/COST851/Default.htm for a detailed list), and this set of techniques is currently being routinely applied to breeding programs in rapeseed, maize, barley and solanaceae crops such as pepper and eggplant. However, this technology is still poorly developed in tomato, mostly due to the extreme recalcitrancy of this crop. In recent years, it has been possible to obtain haploid and doubled haploid plants from in vitro anther cultures, but at a very low frequency (Shtereva et al., 1998; Zagorska et al., 1998; Zagorska et al., 2004; Seguí-Simarro and Nuez, 2005). However, this method poses several drawbacks which prevent it from being efficiently applied on a routine basis. Among these, the most important is the unavoidable presence of somatic diploids coming from the anther walls. This critical issue can be overcome by an in vitro culture of isolated microspores. Very recently, it has been demonstrated that microspore-derived haploid embryogenesis can be achieved in tomato (Dr. J. M. Seguí-Simarro, personal communication), but this technique is still in its infancy and much work must still be devoted to this topic.

8 Integration of New Biotechnologies in Breeding Programs

As advances in biotechnology have been made, they have been applied to tomato breeding. We will review the impact of molecular markers and their applications, transgenics, and more recently genomics on the improvement of tomato.

8.1 Molecular Markers

A great number of molecular markers (RFLP, AFLP, RAPD, CAPs, SSR) are mapped onto the genetic map of tomato. The most recently developed molecular markers are single nucleotide polymorphisms, SNPs (Yang et al., 2004; Yamamoto et al., 2005) and conserved ortholog set, COS markers (Fulton et al., 2002b). Fulton et al. (2002b) screened a large tomato EST database against the *Arabidopsis* genomic sequence and identified a set of 1025 genes (referred to as a conserved ortholog set, or COS markers) that are single or low copy in both genomes.

Molecular markers have been used extensively in tomato breeding. The most important uses have been: the study of molecular variability and phylogenetic relationships, the varietal identification, the marker-assisted selection, the map-based cloning of genes or QTLs, the construction of high-density maps and the construction of mapping populations. A complete review of the applications of molecular markers in plant breeding can be found in Nuez and Carrillo (2000). For a review of molecular markers in tomato, see Foolad and Sharma (2005).

8.1.1 Availability of High-Density Maps

The latest published classical linkage map of tomato consists of 285 known morphological and physiological traits, isozyme markers and disease resistance traits for which the corresponding genes have been mapped onto the 12 chromosomes (Tanksley, 1993).

With the advent of DNA marker technology, the number of mapped markers has increase dramatically (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=tomato), which has led to the development of high-density tomato maps. The first molecular linkage map of tomato contained 94 DNA markers and 18 isozymes (Bernatzky and Tanskley, 1986). Several molecular tomato maps have been developed quite recently (see Foolad and Sharma (2005) for a review), the most important of which are available on the Solanaceae Genomic Network webpage (http://www.sgn.cornell.edu) (Table 9).

All the markers and mapping data for these maps are stored, searchable, and browsable on the SGN database. The relationships between the different genetic maps are also shown. An interactive comparative map viewer allows the comparison of the tomato-potato and tomato-eggplant maps.

Many applications of the high density molecular linkage maps of tomato have been exploited, in particular the identification of molecular markers tightly linked to genes of interest, the positional cloning of certain genes and the detection of the QTLs affecting quantitative characters.

8.1.2 Construction of Mapping Populations

The availability of high-density genetic maps has facilitated the construction of the Substitution Line, Advanced Backcross and Backcross Inbred Line populations, which have facilitated the identification and mapping of QTLs (see Paran (2004) for a review).

Substitution lines (SLs) are produced by backcrossing and marker-assisted selection, so that each line is selected to contain a single marker-defined chromosome segment introgressed from a donor parent in an otherwise uniform genetic background of the recurrent parent. SLs in tomato have been developed by Eshed and Zamir (1995), Eshed et al., (1996) and Monforte and Tanksley, (2000a and b).

The Advanced Backcross QTL (AB-QTL) strategy was developed by Tanksley and Nelson, (1996). AB-QTL enables the discovery and transfer of valuable QTL alleles from unadapted donor lines, such as wild species, into a cultivated species. As

stated by Tanksley and McCouch (1997), "the molecular linkage map is employed to identify the chromosomal position of "wild" alleles that have been transmitted into the progeny, to determine which of the wild species introgressions are associated with superior performance of lines, and to purify lines so that they contain only a specific "wild QTL" in an elite genetic background". This method has been used extensively in tomato (Tanksley et al., 1996; Bernacchi et al*.,* 1998a,b and c; Fulton et al*.,* 1997, 2000; Frary et al., 2004).

Table 9. Molecular maps of tomato.

		Number of markers							
Map (Reference)	**Mapping population**	**CAP**	**SNP**	**RFLP**	**SSR**	**COS**	**KFG**	**Unk-nown**	**TOTAL**
Tomato EXPEN 1992 (Tanksley et al., 1992)	F2 *S. lycopersicum* cv. VF36 x *S. pennellii* LA716	2	-	919	-	2	2	84	1009
Tomato EXHIR 1997 (Bernacchi and Tanksley, 1997)	BC1 *S. lycopersicum* TA209 x *S. habrochaites* LA1777	-	-	134	-	-	-	1	135
Tomato EXPEN 2000 (Fulton et al., 2002b)	F2 *S. lycopersicum* VF925 x *S. pennellii* LA716	699	19	1342	156	544 (523 COSII)	38	-	3321
Tomato EXPIMP 2001 (Tanksley et al., 1996; Grandillo and Tanksley, 1996)	RI *S. lycopersicum* TA209 x *S. pimpinellifolium* LA1589	1	-	143	-	-	1	1	146

Source: http://www.sgn.cornell.edu

Backcross Inbred Lines (BIL) are produced by repeated backcrossing followed by several generations of inbreeding. This method was proposed by Wehrhahm and Allard (1965) to introgress quantitative traits and to estimate their gene number. The BIL method has been used as a breeding method to improve quantitative traits in

tomato (Azanza et al., 1995; Hartman and St. Clair, 1998; Chetelat and Meglic, 1999; Doganlar et al., 2002a; Monforte and Tanksley 2000a).

The availability of high-density maps combined with the development of populations such as Substitution Lines, Advanced Backross QTLs and Backcross Inbred Lines has facilitated enormously the mapping of many genes and QTLs associated with resistance to quantitatively inherited diseases and characters related to fruit quality.

8.1.3 Marker-Assisted Selection (MAS)

A genetic marker can be viewed as a tag attached to a specific segment of a chromosome, which may be associated with a specific phenotype. The use of molecular markers has several advantages compared with phenotypic selection, mainly the speeding up of backcross programs, the elimination of environmental risks derived from the manipulation of pathogens and the elimination of the environmental effect on character expression.

Molecular markers linked to many genes of interest in tomato breeding, mainly genes of resistance to diseases and parameters related to quality have been identified. Although the first molecular markers identified were linked to major genes, today a large number of molecular markers linked to QTLs are known. The molecular markers linked to the main genes of resistance to diseases are indicated in Table 10. A complete review of molecular markers linked to genes and QTLs related to disease resistance can be found in Foolad and Sharma (2005), and for molecular markers linked to QTLs responsible for different parameters related to quality see Gragera (2006).

MAS is routinely used in tomato by seed companies and public researchers to improve varieties for simple traits, mainly resistance to diseases and other plant and fruit characteristics such as plant growth habit (self-pruning), fruit colour and jointless pedicel.

MAS not only accelerates the gene transfer process, but it also allows the pyramiding of desirable genes from different genetic backgrounds in order to enhance the level of the character expression. Additionally, MAS can accelerate the breaking of undesirable linkage associations and rapid recovery of recurrent parent by reducing linkage drag in backcross breeding programs where wild species are used as donors. However, in spite of the effort made recently to identify molecular markers linked to QTLs, MAS for QTLs has been less used. There were value-related problems in the lines derived when MAS was used for QTLs (Foolad and Sharma, 2005). More work is necessary in order to refine the exact locations of QTLs in order to maximize the utility of MAS.

8.2 Transgenics

In tomato, efficient transformation protocols based on *Agrobacterium tumefaciens* and biolistics are available, and their efficiency is increasing continuously (Sun et al., 2006). The first transgenic cultivar, 'Flavr-Savr'^{TM}, was commercialized in 1994, although it did not achieve a remarkable success (Lindhout, 2005).

Table 10. Molecular markers closely linked to genes of resistance used in MAS.

Gene*	Reference
Fungi	
Ve	Kawchum et al., 1994 Diwan et al., 1999
I1, I2, I2-C, I3	Sarfatti et al., 1989; Bournival et al., 1990; Ori et al., 1997; Sarfatti et al., 1991; Segal et al., 1992; Simons et al., 1998; Tanksley and Costello, 1991
Asc	van der Biezen et al., 1995 Mesbah et al., 1999;
Sm	Behare et al., 1991
Cf-1, Cf-2, Cf-4, Cf-5, Cf-9	Van der Beek et al., 1992; Balint-Kurti et al., 1994; Jones et al., 1993
Lv	Chunwongse et al., 1994.
Ol-1, Ol-2	De Giovanni et al., 2004; Huang et al., 2000; van der Beek et al., 1994
Ph-1, Ph-2, Ph-3	Chunwongse et al., 2002; Moreau et al., 1998; Pierce, 1971.
Frl	Vakaluonakis et al., 1997
py-1	Doganlar et al., 1998
Viruses	
Tm-1, Tm-2^2	Levesque et al., 1990; Vakalounakis et al., 1997.
Sw5	Brommenschenkel and Tanksley, 1997; Stevens et al., 1995
Ty-1	Zamir et al., 1994; Pérez de Castro et al., 2007b
pot-1	Parrella et al., 2002
Cmr	Stamova and Chetelat, 2000
Am	Parrella et al., 2000
Bacteria	
Pto, Prf	Martin et al., 1991, 1993a,b (*Pto*); Salmeron et al., 1996 (*Prf*)
Bs4	Ballvora et al., 2001
Nematodes	
Hero	Ganal et al., 1995
Mi, Mi-1, Mi-3, Mi-9	Messeguer et al., 1991; Ho et al., 1992; Williamson et al., 1994; Yaghoobi et al., 1995; Veremis et al., 1999; Ammiraju et al., 2003

Source: modified from Foolad and Sharma (2005).
*See Table 7 to know the pathogens to which the genes included in this table confer resistance

The development of transgenic plants has continued mainly with the introgression of genes related to disease resistance and to fruit quality (Table 11). However, only a few notifications have been approved and none of them have had significant market presence. Transgenic plants have also been developed for scientific purposes, for instance in the restoration of fertility, *Ac/Ds* transposon

system, parthenocarpy and increasing amino acid content in the seed. The transgenic approach to manipulating polygenic characters is much more complex. The genetic transformation of such traits is not yet a straightforward process, although current techniques may allow the transfer of multiple major genes that may act epistatically or additively to improve plant performance.

Table 11. Genes employed in transgenic tomatoes.

Characteristic	Gene	Effect	Reference
Fruit quality			
Less cell wall degradation and fruit softening	Full-length PG cDNA in reverse orientation	Substantial reduction in the levels of PG mRNA and enzymatic activity in ripening fruit. The reduction in PG activity not preventing the accumulation of the red pigment lycopene	Sheehy et al., 1988
Inhibition of fruit ripening	1-aminocyclopropane-1-carboxylate synthase (antisense)	Inhibition of fruit ripening in tomato plants. Administration of exogenous ethylene or propylene reverses the inhibitory effect	Oeller et al., 1991
Fruit sweetness	Single chain monellin gene	Pericarp of transgenic tomatoes tastes sweet	Peñarrubia et al., 1992
Flavour aldehydes and alcohols	Tomato alcohol dehydrogenase (ADH) cDNA (ADH 2)	Significantly higher ADH activity, up to twice that of controls. More intense ripe fruit flavour.	Speirs et al., 1998
	Gene of the thaumatin protein from *Thaumatococcus daniellii*	Better flavour of tomatoes	Bartoszewski et al., 2003
β-carotene	Phytoene synthase gene	β-carotene levels up to 60 μg/g fw in the tomato fruit	Rosati et al., 2000
Carotenoids	phytoene desaturase (*crtl*)	Twofold increase in total carotenoids	Fraser et al., 2001
	phytoene synthase (*crtB*)	Total fruit carotenoids of primary transformants were 2-4 fold higher than the controls, whereas phytoene, lycopene, β-carotene and lutein levels were increased 2.4, 1.8 and 2.2 fold, respectively	Fraser et al., 2002
Parthenocarpy			
Seedless fruit	*Agrobacterium rhizogenes*-derived gene rolB	Parthenocarpic fruits comparable to those of seeded fruits of the parental line	Carmi et al., 2003

Seedless fruit	iaaM gene from *Pseudomonas syringae* pv. *savastanoi*	In transgenic plants the fresh weight of fruits from pollinated or emasculated flowers did not differ from that of fruits obtained by pollination of control plants	Ficcadenti et al., 1999
Seedless fruit	iaaM	Parthenocarpic fruits containing tenfold less seeds that control fruits	Rotino et al., 2005
Constitutive overproduction of antifungal compounds			
Resistance to fungus	class I chitinase and class I β-1,3-glucanase gene	Simultaneous expression resulting in increased resistance to *Fusarium oxysporum* f.sp. *lycopersici*	Jongedijk et al, 1995
Bacterial disease resistance			
Resistance to *Xanthomonas campestris* pv. *vesicatoria*	*Bs2* from pepper	Resistant tomato plants	Tai et al., 1999
Viral disease resistance			
Resistance to Geminiviruses (TYLCV, TYLCSV, TLCV)	*V1* (TYLCV capsid protein) of TYLCV	Delayed disease symptoms and recovery from the disease. Some plants reacted as susceptible	Kunik et al., 1994, 1997
	Truncated *C1* gene of TYLCSV	Plants resistant to TYLCSV, "curled" phenotype	Brunetti et al., 1997
	Eight different constructs of *Rep* and *C4* genes of TYLCV	Transgenic plants with three constructions showed no symptoms and no TYLCV genomic DNA was detected	Yang et al., 2004
	Truncated replication-associated protein (T-Rep) of TYLCV (Mild)	Resistance only against the virus strain from which the truncated T-Rep gene was derived when plants were inoculated by whitefly. Variable response obtained when agroinoculation used	Antignus et al., 2004
Tomato spotted wilt virus (TSWV)	*N* (nucleoprotein gene)	High levels of resistance maintained in hybrids	Ultzen et al., 1995
	N (nucleoprotein gene)	Resistant plants obtained	Gonsalves et al., 1996
	N (nucleoprotein gene)	Highly resistant plants	Nervo et al., 2003
	N (nucleoprotein gene)	Plants protected against virus infection	Fedoro-wicz et al., 2005
Cucumber mosaic virus (CMV)	Satellite RNA, T73-satRNA	Transformants exhibit very slight CMV symptoms, grow normally, and finally set fruits	Saito et al., 1992

CMV Satellite RNA	Transgenic plants show mild disease symptoms followed by a decrease of symptoms	McGarvey et al., 1994
CMV satellite RNA	Mild or no CMV symptoms and low viral titres	Stommel et al., 1998
Chimeric gene expressing a benign satellite CMV RNA	Virus incidence was slightly lower in transgenic plants when compared with controls	Monti et al., 1999 Cillo et al., 2004
cDNA of a symptom suppressor strain of CMV satellite RNA	Transgenic line nº 4-7 show very mild mosaic symptoms when inoculated with virulent CMV isolates with no satellite RNA. Different levels of resistance obtained when inoculated with different strains of CMV	Kawabe et al., 2002
CMV coat protein	Resistant plants obtained	Xue et al., 1994
	Most transformed lines show high level of resistance	Barba et al., 1998
	High levels of resistance found in transgenic plants	Murphy et al., 1998
Defective replicase gene from RNA2 of CMV	Highly resistant transgenic lines obtained	Gal-On et al., 1998
Truncated replicase gene encoded by RNA2 of CMA strain GT, subgroup II	Fifteen transgenic lines (10%) highly resistant to CMV	Nunome et al., 2002
CP gene from the CMV-D strain and Italian CMV isolates of subgroup I and II. Four transformation vectors constructed	All four vectors generate plants with extremely high resistance to CMV	Kaniewski et al., 1999
Trichosanthi, a type-I ribosome-inactivating protein	Transgenic plants show some resistance to *Tomato mosaic virus* and *Cucumber mosaic virus*	Yong et al., 1999

The commercialization of transgenic tomatoes has not been successful due, among other reasons, to the complicated and expensive authorization for commercialization, the great genetic variability available to breeders, the emphasis on qualitative manipulation in altering quantitative traits and the social issues associated with transgenic crops.

8.3 Genomics

There are some important tomato genomics initiatives, including the sequencing of the complete genome and also the comparison to other economically important solanaceous crops. The most important one is the SOL Genomic Network (SGN; http://www.sgn.cornell.edu). The SOL Genomic Network is a comparative resource for the plants of the Solanaceae family, which includes important crops such as tomato, potato, eggplant and pepper. The aim of the SGN is to relate these species to one another using a comparative genomics approach and to tie them to the other dicots through the fully sequenced *Arabidopsis* genome. A high quantity of genomic resources in tomato are available, including collections of transposon-tagged populations (Meissner et al., 2000), mutagenized fast-neutron bombardment populations, EMS-mutagenized populations in Micro-Tom (Meissner et al., 1997) and M82 backgrounds (http://soldb.cit.cornell.edu/mutants_web/) (Emmanuel and Levy, 2002), multifunctional T-DNA/Ds tomato lines (Gidoni et al., 2003), a public cDNA microarray with 12,000 elements representing 8,700 unigenes based on the EST collection which is widely used by the Solanaceae research community (http://bti.cornell.edu/CGEP/CGEP.html), a tomato expression database (TED) (Fei et al., 2006; http://ted.bti.cornell.edu), a physical map (88,642 BAC clones from a *Lycopersicon esculentum* cv. Heinz 1706 BAC library) and an extensive EST collection. Currently, the number of ESTs of tomato is 184,860, representing 30,576 unigenes, 11,160 singletons and 19,416 contigs (Mueller et al., 2005; http://www.tigr.org/tdb/tgi/plant.shtml).

Parallel to the SGN, another initiative, the International Solanaceae Project (SOL), was formed as an umbrella organization for Solanaceae research in over 30 countries to address important questions in plant biology. The first objective of the SOL is the sequencing of the entire euchromatic portion of the tomato genome (Mueller et al. 2005b). The tomato genome is being sequenced by an international initiative of 10 countries: China, France, India, Italy, Japan, Korea, The Netherlands, Spain, The United Kingdom and the United States as part of a larger initiative called "The International Solanaceae Genome project (SOL): System Approach to Diversity and Adaptation (http://www.sgn.cornell.edu/about/tomato_sequencing.pl). Additionally, several countries have their own genomics projects or projects that use genomic tools to address specific questions in Solanaceae.These initiatives have generated a great quantity of information that is being applied to tomato breeding.

8.3.1 Analysis of Large-Scale Microarray Expression Profiles

Arabidopsis thaliana has been used for functional studies of some tomato genes since well before the information generated by the sequencing of the tomato genome

was utilized. Expression of tomato genes in *Arabidopsis* for functional studies has been conducted for genes belonging to the ethylene-receptor family (Tieman and Klee, 1999) and for *Pti4* and *Pti6*, both of which encode *Pto*-interacting proteins (Mysore et al., 2001).

New tomato resources, including the EST database and the microarray technology, have been applied mainly to the study of the ripening process. This has resulted in a cDNA microarray for the study of fruit development and ripening. A time course of ten intervals has been established, spanning fruit development from 7 d postanthesis to 15 d past breaker. Wild types and mutants known to have altered perceptions of light (*hp-1*), ethylene (*Nr*), and other aspects of ripening (*rin, nor*) have also been studied (Giovannoni, 2001; Alba et al., 2005). The resulting data has revealed that although there are defined patterns of differential expression for each developmental stage, there is also a dramatic increase in the number of differentially expressed genes that corresponds to the onset of ripening. The inclusion in the study of the mutant Never-ripe (*Nr*) demonstrated the inhibition of 37% of the genes differentially expressed during the development process of the pericarp, supporting multiple points of ethylene regulatory control during fruit development. As the initial developmental profile has been completed, it may soon be possible to make predictions about ESTs with little or no known homology, based upon their expression patterns and how they relate to genes that have been extensively characterized (Moore et al., 2002).

The correlated expression profiles of 6758 genes across 25 different tomato tissues have also been studied (Fei et al., 2004). Highly expressed genes in various tomato tissues were identified, as were sets of differentially expressed genes associated with plant development with an emphasis on fruit ripening. For fruit ripening, a total of 333 ripening-induced genes and 185 ripening-repressed genes were identified and subdivided into 13 categories according to putative function.

The physiological and molecular responses to salt stress have been studied employing a microarray constructed from Micro-Tom (8700 unigenes), a miniature tomato and TOM1 (Termolino et al., 2006). 731 and 676 EST differentially expressed clones in response to salt stress were identified in roots and leaves, respectively. Many of the genes encoded proteins involved in protein metabolism, signal transduction, primary metabolism and transcriptional regulation. 78 differentially regulated clones were common between leaves and roots.

8.3.2 Comparative Mapping and Genomics

Many comparative mapping studies of Solanaceae were conducted before the DNA sequences generated by sequencing projects were available. Comparative maps between tomato and potato (Tanksley et al., 1992), tomato and pepper (Livingston et al., 1999) and tomato and eggplant (Doganlar et al., 2002b) have established the syntenic relationships between their genomes.

Useful information has been generated regarding the phylogenetic relationships and main rearrangements that occurred in the genome during the evolution of tomato, potato and pepper, the evolution and conservation of the functions of different types of R genes and also of recessive resistance genes, the number of loci

implicated in the drastic phenotypic changes observed in the domestication process from wild relatives in tomato, pepper and eggplant and the comparison of the function of genes affecting fruit characteristics (Table 12).

Table 12. Comparative mapping in Solanaceous crops.

Crops	**Molecular markers or genomic sequences**	**Results**	**References**
Phylogenetic studies			
Tomato and potato	RFLPs	5 paracentric inversions	Bonierbale et al., 1988; Gebhardt et al., 1991; Tanksley et al., 1992
Tomato and pepper	RFLPs	Substantial rearrangements, loss of homologous regions	Tanksley et al., 1988; Prince et al., 1993
Pepper, tomato and potato	RAPDs, isozymes, AFLPs; RFLPs	Eighteen homeologous linkage blocks cover 98.1% of the tomato genome and 95% of the pepper genome. 5 translocations, 10 paracentric inversions, 2 pericentric inversions and 4 disassociations or associations of genomic regions differentiate tomato, potato and pepper	Livingston et al., 1999
Tomato and *Arabidopsis*	A 105-kilobase bacterial artificial chromosome (BAC)	The tomato clone shows conservation of gene content and order with four different segments of *Arabidopsis* chromosomes 2-5	Ku et al., 2000.
Tomato and eggplant	RFLPs	Conservation of large tracts of collinear markers. Tomato and eggplant were differentiated by 28 rearrangements, which can be explained by 23 paracentric inversions and five translocations	Doganlar et al., 2002b
Phenotypic changes during domestication			
Tomato, pepper and eggplant	Quantitative trait loci (QTLs)	The phenotypic diversity of the three crops is likely to be the result of differential	Doganlar et al., 2002c

		transcriptional regulation of similar gene sequences. Few loci are implicated in the drastic phenotypic changes observed in the domestication process from wild relatives in tomato, pepper and eggplant	
Genes of resistance			
Tomato, potato, pepper	All published chromosomal locations for R genes and major QRLs for tomato, potato and pepper	While the taxonomic specificity of host R genes may be evolving rapidly, general functions of R alleles (initiation of resistance response) may be conserved at homologous loci in related plant genera	Grube et al., 2000
	Genomic sequences from tomato showing significant homology to genes conferring race-specific resistance to pathogens	Phylogenetic clustering of R-gene homologues between tomato and other Solanaceae family members was observed but not with R-homologues from *A. thaliana*. This shows the rapid evolution of R-gene homologues during diversification of plant families	Pan et al., 2000.
Tomato and pepper	Comparative genetics of resistance to potyviruses: genes of resistance *pvr1, pvr2, pvr3, Pvr4, pvr5, pvr6* and *Pvr7* in pepper and *pot-1* in tomato	Orthology exists between *pot-1* from tomato and the recessive locus *pvr2/pvr5* from pepper, indicating that they evolved less rapidly than the majority of the dominant genes studied so far	Parrella et al., 2002
Fruit characteristics			
Tomato and pepper	Structural genes from the *Capsicum* carotenoid biosynthetic pathway, *C2, Ccs, Y,* lycopene β-cyclase gene and the lycopene ε-cyclase locus,	Associations and correspondences were established between the CSS, lycopene β-ciclasa and lycopene ε-ciclasa genes of pepper and the *B, lutescens-2* and lycopene ε-ciclasa genes of tomato, indicating that fruit colour variation in tomato and pepper is partially controlled	Thorup et al., 2000

have been compared with positions of the same genes in tomato	by corresponding genes. The comparative analysis using candidate genes has shown to be useful in linking specific metabolic phenotypes and loci that affect these phenotypes in related species	
Twelve and 31 fruit weight QTLs and 7 and 21 fruit shape QTLs in pepper and tomato, respectively	Twenty-seven percent of fruit weight QTLs were conserved in both genera; all fruit shape QTLs were unique in each genus	Paran et al., 2004
Fruit weight QTLs*: fw2.1, fw2.2*, *fw4.1* and *fw4.2* and one fruit shape QTL*: fs2.1*	The *fw2.1* fruit weight QTL and the *fs2.1* fruit shape QTL of pepper were localized to the tomato fruit shape gene *ovate*. Co-localization of the pepper QTLs with QTLs identified for similar traits in tomato suggests that the pepper and tomato QTLs are orthologous	Zygier et al., 2005

Extensive sequence information is currently only available for *Arabidopsis* and rice, although international efforts have been initiated for tomato. These sequence resources that are already available can be exploited for discovery in species such as tomato, for which less information and fewer resources are currently available. Thus, the sequenced genome of *Arabidopsis* and the large numbers of EST sequences already available in tomato have made possible the identification of a set of 1025 genes (conserved ortholog set, or COS markers) that are single or low copy in both genomes. This gene set can be used for comparative mapping studies between highly divergent genomes such as those of tomato and *Arabidopsis* (Fulton et al., 2002b), thus avoiding problems of weak hybridization signals usually found when *Arabidopsis* genes are hybridized with tomato genomic DNA.

ESTs can be used for gene discovery and expression analysis. The development of EST and microarray technologies makes it possible to incorporate transcriptional information into comparative genomic studies. Starting from the existence of the large number of ESTs available not only for tomato but also for other species, Fei et al. (2004) studied the correlated expression profile of 6758 genes across 25 different tomato tissues. They compared the highly expressed genes in various tomato tissues with the corresponding genes in *Arabidopsis* and grape. They also identified sets of differentially expressed genes associated with plant development with an emphasis on fruit ripening. The comparison of the available grape ESTs allowed the identification

of a set of transcription factors induced during both tomato and grape ripening that had not previously been associated with ripening.

Another study using microarray technology for interspecies comparison of gene expression was conducted by Alba et al (2004). They co-hybridized labelled DNA populations derived from pepper and tomato pericarps (both from breaker-stage fruit) to the TOM1 microarray. Pepper genes showing increased transcript abundance in this experiment (compared with expression in equivalent tomato tissue) are of particular interest, as this result cannot be explained by differential hybridization because of sequence divergence.

Expression profiling studies of fruit ripening and development between tomato, pepper and eggplant have also been conducted using the tomato cDNA microarray (Moore et al., 2005). The results of this study indicate that, while many transcripts are commonly expressed in fruit development among the species tested, several divergent mechanisms are at play when comparing tomato and pepper with eggplant, specifically the transcripts involved in plastid structure and photosynthesis. While the ripening expression profiles of tomato and pepper share more similarities compared to eggplant, significant differences were detected.

8.3.3 Targeting Induced Local Lesions in Genomes (TILLING) and EcoTILLING

Tilling is a high-throughput PCR-based screening technique that induces DNA variation in M2 populations. By using this technique, Hurst et al. (2006) identified reduction-of-function alleles of the endogenous polygalacturonase enzyme, which breaks down cell wall pectins during fruit ripening. The detected mutations severely affect enzyme activity, which is of great interest both for fresh and processed tomatoes. The application of the TILLING technique to germplasm collections (EcoTILLING) is going to be performed as part of the European EUSOL initiative.

9 Seed Production

Tomato is considered a strict autogamous plant with natural self-pollination varying from 94% to 99%. The percentage of allogamy varies depending on the cultivation type (plastic-house or open air), the presence of pollinating insects, frequency and intensity of winds, presence of fences, relative humidity and other factors. However, in warm areas, it usually varies between 0 and 5%, with much higher values possible in tropical areas. In those conditions that most favour crosspollination in seed production assays, it is of interest to cover the different varieties with anti-insect screens or to separate the plants by a safety zone. However, the multiplication of homozygous materials and the derivation of pure lines usually do not require special isolation measures.

It is necessary to take care with the regeneration trials as the stresses produced by scarce nutrients can reduce the set of the fruits and the quantity of seeds per fruit. Fertilization with a balance of N, P and K increases seed production (Arya et al., 1999). Seed production also depends on genotype (Cheema and Dhaliwal, 2004).

Hybrid seed can be produced by several methods:

-A) hand emasculation and hand pollination.
-B) use of male sterility followed by hand pollination.

A) Hand emasculation and pollination is the most expensive and time-consuming method. However, due to the high price of hybrid seed, it is the most used. Emasculation should be performed when petals have just opened and form a 45º angle with the axis of the flower, eliminating stamens with the aid of clamps. The pollen is taken from a fully mature flower and placed on the stigma of the flower emasculated two days before, when the female flower became fully receptive. Repeated pollinations have been shown to improve the efficacy of the results (Zhu et al., 2002). The storage of pollen for five days at room temperature does not affect its viability. Pollen stored under refrigerated conditions (constant temperature of 9-10 ºC) does not show a significant drop in viability even after seven days of storage (Yogeesha et al., 1999). Cryogenic storage can be applied to pollen. Flowers pollinated with pollen samples stored for five weeks at –80 ºC had similar fruit set and number of viable seeds per fruit as those pollinated with fresh pollen. The same results were obtained when the pollen was repeatedly cooled and warmed in up to six cycles (Sacks and St. Claire, 1996). After pollination, covering the pollinated flower is recommended if the process is done in the open air in order to protect the female flower from foreign pollen. A paper bag is the most suitable material for this operation.

B) Use of male sterility and hand pollination.

Three types of male sterility in tomato are known, and all of them are governed by single recessive genes: a) the long series of *ms* genes produce sterile pollen. All of these genes are recessive except *MS-48,* which is dominant. This type of sterility is maintained though backcrossing (*ms ms* x *Ms ms*). To facilitate the elimination of 50% of the fertile plants (*Ms ms*), the morphological marker gene "anthocyaninless" (*aa*), which is closely linked to the *ms*10^{35} gene, can be used. However, the efficiency of this method depends on the strength of the linkage between the two genes. b) Stamenless genes (*sl*) produce flowers with only a vestigial development of the stamens which under most conditions produce no anthers or pollen (Bishop, 1945). c) Functional male sterility mutants, which include *positional sterile* (*ps*), *positional sterile 2* (*ps-2*), *cleistogamous 2* (*cl 2*), *dialytic* (*dl*), and *exserted stigma* (*ex*) (Gorman and McCormick, 1997; Atanassova, 2000). Of all these, the most studied is the *ps-2* gene. The *ps-2* gene resulted from a spontaneous mutation in the Czech cultivar Vrbicanske nizke and is characterized by the presence of fertile pollen and indehiscent anthers (Tronickova, 1962). Testing *ps-2* lines under a large range of environmental conditions showed that occasionally the retention of the pollen was not totally consistent. However, further studies have shown that it is possible to improve the efficiency in the use of this type of sterility. Even in this case, the emasculation would be conducted at anthesis, which is significantly easier than in floral buds, making the utilization of this type of sterility more viable in hybrid seed production. *ps-2* sterile parents have been used in a few countries: the Czech Republic, Moldova, Poland and Bulgaria, and a number of hybrids possessing functional male sterile seed parent have been released (Atanassova, 1999, Atanassova and Georgiev, 2002). *Exserted stigma* sterility (*ex*) is not associated

with alterations in the anthers as their dehiscence is normal and they produce viable pollen. It is instead due to the excessive length of the style, which protrudes from the cone, holding the stigma out and away from the concentration of pollen in the central space and which effectively produces a functional male sterile phenotype (Rick and Robinson, 1951). However, its expression depends on the lengths of both style and anther, these lengths being polygenically controlled, and varying significantly depending mainly on the temperature (Georgiev and Atanassova, 1977). The conclusion drawn from this and other problems associated with this type of sterility was that the ex-sterility would be rather inapplicable in hybrid seed production.

The most used genes for producing hybrid seed are the *ms* series and the functional sterility controlled by the *positional sterility 2* gene.

In spite of the significant amount of research on the application of genic male sterility in tomato breeding, up to now, male sterile lines have not been used on a large scale in tomato hybrid seed production.

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Carrot

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1 Introduction

Carrot is among the top-ten most economically important vegetable crops in the world, in terms of both area of production and market value (http://faostat.fao.org/faostat/; Rubatzky et al., 1999; Simon, 2000; Fontes and Vilela, 2003; Vilela, 2004). In 2005, world production approached 24 Mt on 1.1 million hectares. The total global market value of the more widely traded carrot seed crop has been estimated to be in the range of $100 million (Simon, 2000), but such estimates have little reliable data to confirm them and true value is likely much more. The development of cultivars adapted for cultivation in both summer and winter seasons on all continents has allowed a year-round availability of carrot products with relatively stable prices to consumers. Some production areas harvest crops year-round.

Carrot improvement today includes several academic, private and government research programs around the world that work in concert with local, regional, and global industries. Both grower and consumer needs are addressed by public and private carrot breeders that incorporate modern technologies into the classical breeding process.

2 Origin and Domestication

Carrot is the most widely grown member of the Apiaceae or Umbelliferae. This diverse and complex plant family includes several other vegetables, such as parsnip, fennel, celery, root parsley, celeriac, arracacha, and many herbs and spices (Rubatzky et al., 1999). Like other plants of this family, carrot seeds are aromatic and consequently have long been used as a spice or herbal medicine. In fact, carrot

seed was found in early human habitation sites as long as 3000 to 5000 years ago in Switzerland and Germany (Laufer, 1919). This seed is thought to be from wild carrot used for flavour or medicine.

The genus *Daucus* includes approximately 20 species scattered worldwide but the majority of species occur around the Mediterranean (Saenz Lain, 1981). In contrast to other members of the genus, carrot, *Daucus carota* L., originated in Central Asia. Wild carrot had obviously spread well beyond this region long ago but the use of carrot as a root crop only dates back about 1100 years to its centre of diversity, Afghanistan. Mackevic (1929) described the Afghan carrot and its spread both west and east. The spread of cultivated carrot westward is relatively well-documented, recorded in Persia in the 900s, Middle East and North Africa in the 1000s, Spain in the 1100s and Northern Europe in the 1300s (Banga, 1957a, b, 1963). Turkey is regarded as a secondary centre of diversity for carrot with a long history of diverse carrot types and uses even today. The spread of cultivated carrot eastward is not as well documented as that to the west, but history records its use beginning in China in the 1300s and Japan in the 1700s.

The genetic improvement of carrot has been an ongoing effort throughout its cultivation and domestication. Before the 20th century, carrot production was small-scale in family or community gardens. A portion of the crop was likely protected in the field over winter with mulch, or the best roots saved in cellars were replanted the subsequent spring to produce a seed crop. There is no written record of what traits were evaluated or any other detail of the selection process in this period, but all domesticated carrot differs from its wild progenitors in forming larger, smoother storage roots, so it is clear that these traits also were improved through regular selection. Selection for low incidence of premature flowering was also necessarily among the most important traits selected during domestication, as it is now, since with the initiation of flowering, eating quality diminishes dramatically.

We can surmise that colour and flavour were primary selection criteria since these traits were used to distinguish among carrots recorded by historians, cooks, and eventually seed catalogues. Carrot root colour also changed dramatically during domestication. While wild carrot roots are white or very pale yellow, purple and yellow carrots were the colours of the first domesticated carrots. These were the only colours recorded until the 16th to 17th century when orange carrots were first described and soon came to be preferred in both the eastern and western production areas (Banga 1957a, b, 1963, Rubatzky et al. 1999, Simon, 2000). Banga compiled an extensive list of comments about carrots over history and while purple carrots were usually (but not always) regarded as better flavoured than yellow, the dark stains they left on hands, cookware, and in cooking water raised negative comments by some authors. We do not know why early carrot breeders shifted their preference to orange types, but this preference has had a significant effect in providing a rich source of vitamin A, from alpha- and beta-carotene, to carrot consumers ever since. Soon after orange carrots became popular, the first named carrot cultivars came to be described in terms of shape, size, colour, and flavour, and the first commercially sold carrot seed included reference to this growing list of distinguishing traits.

Carrot domestication took somewhat differing paths east and west of Central Asia, carrot's centre of diversity (reviewed in Banga, 1963; Heywood, 1983).

Before the first reports of orange carrots, purple root colour was apparently more popular in eastern regions, yellow more popular in the west. Eastern carrots tend to have less deeply divided leaflets with heavy leaf pubescence in some cultivars. While early flowering is unacceptable for any carrot production, eastern carrots have a greater tendency toward early flowering than western carrots, likely due to the somewhat warmer climates over the eastern production range. Beyond the yellow, purple, and orange root colours, eastern carrots have long included red-rooted types while western carrots included white-rooted types. Carrot use has also varied across production areas, with a more predominant use as animal forage in the east but largely human use as a root vegetable in the west.

3 Varietal Groups

The first carrots were separated into two groups based upon root colour: purple and yellow. With the development of orange carrots in the west, shape and size also became distinguishing characteristics of carrot types, starting with the description of "Long Orange" and shorter "Horn" types in the 16th century (Figure 1). Numerous variations of both types were also soon described. Differences among named cultivars were noted not only in storage root length but also colour intensity, core colour and size, root tip shape, and rate of crop maturity. Those same distinctions are still used to help categorize carrot varietal groups today. The major root types used today include such varietal groups as European 'Nantes', 'Chantenay', 'Danvers', 'Paris Market', 'Flakkee', 'Berlicum', and 'Amsterdam Forcing', Asian 'Kuroda', North American 'Imperator' and South American 'Brasília'. Numerous cultivars have been named for all of these root types. As new cultivars are developed in colours other than orange, new cultivar groups will likely evolve.

Underlying varietal distinctions based upon storage root colour and shape is adaptation to cool versus warm growing temperatures. Carrot is categorized as a cool-season vegetable and the majority of effort on carrot breeding has been towards improving production in temperate regions where cool temperatures (< ~10C) can stimulate early flowering or "bolting". More recently there have been successful efforts in broadening the adaptation of carrot to warmer subtropical climates where excessive heat can retard plant growth, inhibit root colour development, and stimulate the development of strong flavour in unadapted germplasm. The 'Brasília' cultivar, for example, grows successfully in agricultural regions near the Equator. The development of temperate (late-flowering) and subtropical (early-flowering) types has resulted from a greater emphasis on ability to withstand early bolting in cooler climates for temperate types, in contrast to a greater emphasis on the ability to produce a marketable crop in warm climates for subtropical types. Subtropical carrots tend to grow faster than temperate types suggesting a complex interaction between root growth, flowering induction, and temperature that is not well understood. It should be noted that, unlike many crops, there is little evidence for a photoperiod effect on carrot root production and flowering so that the same cultivar theoretically could be grown anywhere in the world, if temperature requirements are met. In fact, many carrot cultivars are widely adapted and can be grown over such

extreme production temperatures as represented by north of the Arctic Circle to highland subtropical climates.

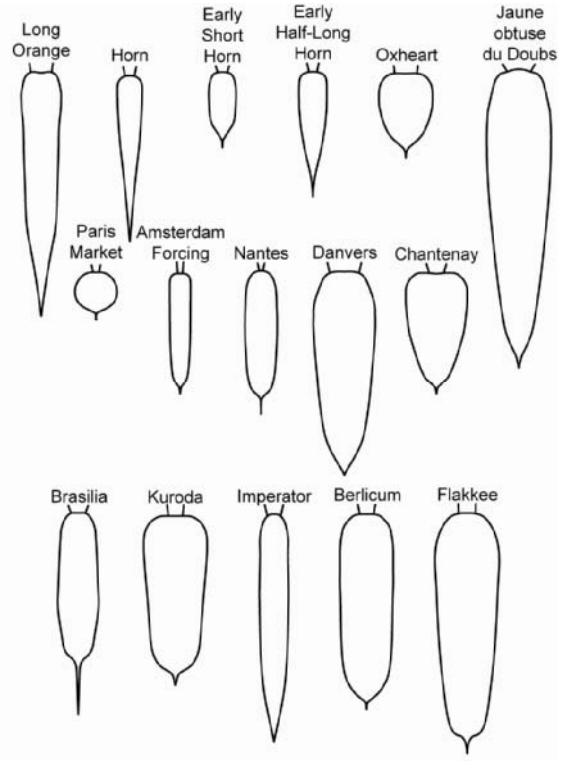

Fig. 1. Typical Carrot Root Shapes. Top row includes original European types; bottom two rows are major types grown worldwide today.

4 Genetic Resources

As for most vegetables, carrot genetic resources held in existing collections (Table 1) focus predominantly upon open-pollinated cultivars. No estimation or assessment has been made to determine if unique germplasm has been lost but recent germplasm-collecting expeditions have revealed that local land races of carrot in use as recently as several decades ago are no longer being maintained by growers in the Middle East and Central Asia.

The U.S. Root and Bulb Vegetable Crop Germplasm Committee makes recommendations regarding the collection, increase, evaluation and distribution of carrot germplasm in the USDA, ARS collection. Since 2000, a European Cooperative Programme for Crop Genetic Resources (ECP/GR) umbellifer group has been active to coordinate European genetic resources management. New procedures

for germplasm characterization, evaluation and reporting have been developed from both genetic resource oversight groups. The U.S. and European databases are on direct access from the internet for both collections (Table 1).

Table 1. Carrot germplasm collections.

Daucus Germplasm Collection - USDA, Agricultural Research Service http://www.ars-grin.gov/npgs/ Taxa: 13 species and 11 *D. carota* subspecies *D. carota:* 723 cultivars, landraces and wild carrot Other *Daucus*: 70 accessions over 12 species, including several unidentified Total: 793 accessions Seed available
Daucus Germplasm Collection - ECP/GR Umbellifer Database http://www2.warwick.ac.uk/fac/sci/hri2/about/staff/dastley/gbrhrigru/ecpumbel/ Taxa: 7 species and 13 *D. carota* subspecies *D. carota:* 5037 cultivars, landraces and wild carrot Other *Daucus*: 279 accesssions over 8 species, including several unidentified Total: 5316 accessions Seed available
Carrot Breeding Collection - National Center for Vegetable Crops Research (CNPH), Empresa Brasileira de Pesquisa Agropecuária (Embrapa Vegetable Crops), Brazil Taxa: *Daucus carota* European, North American, Japanese cultivars /populations and Brazilian cultivars and South Brazil landraces evaluated for heat tolerance and field resistance to the leaf blight complex (*Alternaria dauci*, *Cercospora carotae* and *Xanthomonas campestris* pv. *carotae*) and root-knot nematodes (*Meloidogyne incognita* race 1 and *M. javanica*). Total: 200 accessions Seed availability based upon inquiry
Daucus working collection BAZ - Institute of Horticultural Crops, Germany Taxa: 5 species, 25 subspecies, 30 wild relatives A collection of alloplasmic lines derived from wild x *D.c.* subsp. *sativus*, Several cms – lines Genetically defined material and mutant lines (e.g. *yellow leaf* or *cola*) Seed availability based upon inquiry
Carrot germplasm collection - National Gene Bank, Rural Development Administration (RDA), Korea http://genebank.rda.go.kr Taxa: *Daucus carota* Japanese, European, Russian, Chinese, North American cultivar/populations, Korea cultivars and landraces. *Daucus carota* : 695 accessions. Seed available

An evaluation of genetic diversity in cultivated and wild carrot based on molecular markers indicates a broad range of variation in *Daucus carota* L. (Bradeen et al., 2002). In this study, cultivated and wild carrot were usually in separate clades

with relatively little overlap. Plant-to-plant variation within an open-pollinated cultivar can be quite substantial for molecular markers even if intra-cultivar variation for phenotype is relatively small (Westphal and Wricke, 1991; Schulz et al., 1994; Le Clerc et al., 2005). With this wide range of variation in carrot, relatively large populations are necessary to assure maintenance of allelic diversity (Le Clerc et al., 2003).

5 Major Breeding Achievements

5.1 Historical Progress

As horticulture and plant breeding entered the realms of academia and government research institutes in the late 1800s and early 1900s, descriptions of carrot production came to describe ideal carrots, and breeders began selecting for these ideals. Breeding methods also became more sophisticated in this period. Published works on carrot breeding in the early 1900s described methods for isolating flowering plants from undesired pollen sources, inbreeding and outcrossing (Borthwick et al., 1931; Borthwick and Emsweller, 1933). With the widespread occurrence of wild carrot, isolation of the commercial seed crop from this pollen source is not trivial, and it is critical to the value of that crop. By the end of the 1940s, the success of breeding, selection, and hybridization in improving maize yields was already well-known but the revolutionary discovery of cytoplasmic male sterility (CMS) in onion by Henry Jones in the 1930s was even more important in inspiring a generation of vegetable breeders to develop analogous programs to improve other vegetable crops, including carrots. Thus there was a significant emphasis on first discovering a CMS system for carrot and then applying that system to develop carrot as a hybrid crop.

5.1.1 Carrot Breeding History in the United States

U.S. carrot breeding was initially based entirely on European carrot types with at least 15 seed catalogues selling carrots in the late 1700s into the 1800s. A detailed account of U.S. carrot breeding history has recently been published (Simon and Goldman, 2006). The ‘Long Orange’ and ‘Horn’ types were central to the first U.S. carrots (Figure 1). By the 1800s, short orange, yellow, white, and purple carrots were also available in seed catalogues and grown to some extent, although long and intermediate orange types apparently dominated the U.S. market. Based upon seed catalogue entries, by the mid-1800s purple carrots were no longer routinely available to U.S. growers and yellow carrots became less widely available by the late 1800s. ‘Danvers’ appeared in the U.S. as a cultivar name in the 1890s and in the 1920s a new type, ‘Imperator’, was developed in the U.S. by Associated Seed Growers as a longer, thinner carrot for use in bunching.

With the discovery of cytoplasmic male sterility in carrot by Welch and Grimball (1947), carrot breeding programs were established at 10 U.S. universities and one federal program in the 1950s and 1960s. Hybrid ‘Imperator’, ‘Danvers’, and

'Chantenay' cultivars soon were developed and most of that effort has been taken over by industry breeding programs over the last 20 years.

'Imperator' type cultivars have been the mainstay of U.S. commercial production since the 1950s. The strong tops, clean attachment and tapered storage root made the type very conducive for bunching, mechanical harvesting and use in semi-arid production sites. The 'Imperator' style has proven to be very adapted to the shift in market uses over the years; from the bunching of the 1950s, to the cellophane bag packaging "cello" of the 1960s to present and the fresh cut and peel "baby" style from the 1990s to present.

Open pollinated cultivars such as 'Imperator 58', 'Waltham Hi Colour' and 'Gold Pak' were the predominant choices up until the early 1980s. Various selections and strains of these were maintained by the seed companies. With the discovery of a stable "petaloid" male sterility system in wild carrot in 1953 in the Northeast US by Henry Munger (reported by Peterson and Simon, 1986) and subsequent transfer of that trait into commercially useable inbred lines by individuals such as C.E. Peterson and W.H. Gabelman, commercial hybrids from universities and industry began to show up in large numbers by the early 1970s. By the mid 1980s, most of the carrot production in the U.S. and Canada used 'Imperator' hybrids for fresh market and this type now accounts for 85% of the U.S. carrot crop by production area, and 95% by crop value (http://www.usda.gov/nass/pubs/).

By the early 1980s, private investment in agricultural technology was rapidly increasing and seed company breeding programs, including carrots, were greatly expanded. With better understanding and appreciation of quality attributes in carrots such as carotene content, flavour and sugars, newer 'Imperator' hybrids began to incorporate consumer quality traits into hybrids that also had agronomically sound traits to benefit the grower.

When the new produce phenomena of fresh cut and conveniently packaged vegetables began to grow in the late 1980s and early 1990s, carrots and lettuce were at the forefront of this market. The California carrot production industry developed a high value "cut and peel" or "baby" carrot product using higher quality, more slender 'Imperator' type hybrids that was immensely popular at retail. Mostly due to this new convenient way to purchase fresh carrots, per capita consumption grew significantly in a matter of a few years, accounting for a significant portion of the fresh carrot market today. Much of this increase was due to the increased consumption by children. Now over 15 years later, a new generation of consumer has emerged that prefers and is most familiar with this way to purchase carrots in North America.

Organic production of commercial carrots in California has grown very rapidly since 2000, with a large amount of product delivered throughout the North American retail market. The industry has found that several available hybrids work well for organic production in the semi-arid western U.S. as carrots. As in conventional production, the main criteria in organics are good eating quality, solid disease resistance and economical yield. Carrot use has also expanded as a key component in juice mixtures. Interest by consumers, growers, and the seed industry in "unusual" coloured yellow, purple, red, and white carrots may provide an avenue for the development of other new markets.

In contrast to the larger numbers of U.S. universities involved in carrot breeding several decades ago, relatively few remain today. The U.S. carrot seed industry included a mixture of smaller and larger companies until recent decades when it became consolidated into a few larger programs that address both regional and global markets, as has been the trend in most vegetable crops around the world.

5.1.2 Carrot Breeding History in Brazil

The beginning of carrot cultivation in Brazil and the initial establishment of the Brazilian carrot germplasm coincided with the European immigration to the extreme South of Brazil in the 17th century. The carrot germplasm was introduced by European immigrants from the Azores Islands of Portugal and their descendants have maintained these landrace populations until today. This germplasm went through intentional as well as natural selection cycles with many accessions still being found as feral populations in the South of Brazil where environmental and climatic conditions (temperature and daylength) can induce carrot flowering. The majority of these Brazilian landraces combine one or more of the following characteristics: orange-root colour, heat tolerance, earliness and high levels of leaf blight and root-knot nematode resistance.

Before the development of tropical-adapted cultivars, the predominant varietal groups in Brazil were European 'Nantes'- type (cylindrical root) and the Japanese 'Kuroda'-type (conical root) (Figure 1). The cultivation of these varieties was restricted to South and Southeast Brazil. In the late 1950s, the University of São Paulo State (ESALQ/USP) initiated the first Brazilian carrot breeding program with the major aim being the adaptation of this crop to Brazilian conditions with a focus on *Alternaria* leaf blight resistance (Costa et al., 1974). 'Tropical' and 'Cenoura Nacional' were the most important releases of this era but with little commercial impact. 'Cenoura Nacional' had improved levels of heat tolerance and field resistance to *Alternaria* leaf blight but yet with unsatisfactory root colour (Costa et al., 1974). In 1978, a formal cooperative effort was established between Embrapa Vegetable Crops and ESALQ/USP. This work resulted in the second generation of tropical-adapted, disease resistant cultivars that was released in the early 1980s. The two most important cultivars of this era were 'Kuronan' (selected from 'Kuroda Gosun' *x* 'Nantes' crosses) (Ikuta et al. 1983) and 'Brasília' (Figure 1) derived from the combination of distinct germplasm including 'Cenoura Nacional', 'Tropical' and other South Brazilian landraces as basic breeding germplasm (Vieira et al., 1983).

'Brasília' was the most successful cultivar and soon after its release it became the leading commercial variety, representing around 75% of total Brazilian carrot production today (Vilela, 2004). This was mainly due to its high-yielding potential, heat tolerance and high levels of resistance to the leaf blight complex allowing cultivation during the rainy/hot season with very low agrochemical input (Boiteux et al., 1993). After the release of 'Brasília', carrot cultivation more than doubled between the 1980s and the early 2000s allowing expansion of the crop to new horticultural frontiers in Northeastern Brazil. In addition, the use of this cultivar together with improvements in the crop management system was responsible for a large portion of the spectacular increase of over 150% in productivity observed in the

decades following its release. 'Brasília' and populations derived from it are in the catalogues of all national and international seed companies present in Brazil and this germplasm has been commercialized throughout South America.

After the release of the cultivar 'Brasília' in the early 1980s (Vieira et al., 1983), the carrot varieties were divided into two major groups: summer/fall and winter cultivars. The major varietal group used for carrot production during winter is derived from the European open pollinated 'Nantes' types, but hybrids are being developed. Open pollinated cultivars of the 'Brasília' group are predominant during summer/fall in the South and Southeast growing areas but they are also used year-round in the Northeast, Center-West and North regions. New cultivars derived from 'Brasília' such as 'Alvorada' (Vieira et al., 2000) combining adaptation to tropical conditions with superior root colour, higher total carotene content, and disease (especially nematode) resistance (Vieira et al., 2005b) are able to set the stage for a further expansion of carrot production in warmer climates.

The consumption of minimally processed "baby-carrots" is still very low in Brazil due to their high cost, but the development of tropical-adapted high-carotene/high sugar cultivars would provide the basis for the expansion of the cut and peel processing industry in the country. Embrapa Vegetable Crops has recently released 'Esplanada' to address these needs (Vieira et al., 2005a). 'Imperator' types are also being used. These high-value-added items would provide these regions with new opportunities for economic diversification and growth.

5.1.3 Carrot Breeding History in Europe

The evolution of carrot cultivars in Europe has been ongoing for over 400 years (Banga, 1963) during which several hundred local races and more widely grown open-pollinated cultivars of carrot were selected. Before carrots were transported from distant production regions to meet market needs year-round, two early fresh market types that differ in root length were developed: short, round (5-10 cm) 'Paris Forcing' types and early "half-long" (10-15 cm) 'Oxheart' types for spring and summer production (Figure 1). Short, round cultivars were grown widely by market gardeners in cold frames and sold in bunches until the mid-1970s. "Half-long" cultivars with bolting resistance are very well adapted for planting in cold frames in the autumn and winter sowing. 'Early Half-Long Horn', developed in 1763 was the first true half long variety. Other popular varieties are noted for their earliness, high yield, darker colour and resistance to bolting and root cracking.

For autumn and winter production of carrots for fresh market, 'Nantes' types (15-20 cm, long; 25 - 40 mm diameter) are preferred. 'Nantes' hybrids are now grown year around and represent the majority of carrot type used for fresh market packaging throughout Europe. 'Amsterdam' bunching hybrids represent the other main category for fresh consumption. The strong emphasis on 'Nantes' types began in 1977 with the release of the two first hybrid cultivars 'Tancar' for spring production and 'Nandor' for autumn and winter production. This was followed by many other hybrids and carrot production changed from small-scale market gardens to industrial producers. Relative to the open-pollinated cultivars they replaced, these modern hybrids brought much more homogeneity to the European carrot crop, as

well as adaptation to mechanical harvesting. Hybrids and mechanization reduced the labour required for carrot production. The conversion to mechanized large-scale hybrid carrot production in Europe, as elsewhere in the world, has not only increased production area, but also improved productivity, improved earliness, better shape, reduced incidence of cracking and splitting, and resistance to frost and bolting. In recent decades the focus has broadened to include improvements in foliage quality and root colour. To achieve this purpose, the traditional European type 'Nantes' has been crossed with 'Kuroda' and 'Imperator' types (Figure 1). The first hybrid partially resistant to *A. dauci* was 'Bolero' released in 1991 by Vilmorin. Today, over 90% of registered carrot varieties in France and most of Europe are hybrids (listed in the Common Catalogue of Varieties of Vegetable Species at www.europa.eu.int/eur-lex/en/index.html.). New research focus includes the development of carrots for organic production, and colour diversity for niche markets for when cultivars targeting these markets have been developed.

Carrots for processing (canning and freezing) and overwinter storage account for 20% of the total market. Derivatives of 'Long Orange' including 'Flakkee' and 'Berlicum' that produce a long, dark orange root are typically used for freezing. Shorter 'Amsterdam' types and even the very short 'Paris Market' types are used for canning and bunching (Figure 1). A very small portion of the carrot production today is used for animal feed. These are typically very large yellow or white cultivars including 'Jaune Obtuse du Doubs' and 'Blanche à Collet Vert Très Hors Terre'. Most of the carrots used for processing and animal feed are open-pollinated.

The first carrot breeding companies in Europe included Vilmorin (established 1742) and Clause (1890) in France; Mette (1784) and Sperling (1788) in Germany; Widow S. Groot (1830) and Slius and Groot (1867) in the Netherlands; James Carter (1841) in England; and Ulrich (1805), Hoser (1848) and Freege (1860) in Poland. Carrot research at European government institutes and universities began in the 1940s.

5.1.4 Carrot Breeding History in Asia

Original Asian carrots came from Central Asia through China in the early Yuan Dynasty (1280-1367) (Laufer, 1919) and these spread from the south to central and northern regions and then on to Japan in the 1600s and Korea in the 1900s. European carrots also have been grown and adapted for Asian production for hundreds of years in China. Early carrot breeding in Japan (Shinohara, 1984) led to two types derived directly from central Asian carrot: the very long (sometimes exceeding 80 cm) pale orange 'Takinogawa' type that is very rare or extinct today, and the red 'Kintoki' that is still grown. European 'Long Orange', 'Early Half Long Horn', 'Early Short Horn', and 'Chantenay' types were adapted for cultivation across the various climatic and production regions of Japan since the 1700s. In the 19th and 20th centuries Japanese breeders used derivatives from these starting materials to develop several major types including dark orange 'Kokubu', pale to dark orange, heat-tolerant 'Gosun', and orange, non-bolting 'Sanzun'. A dark orange selection from 'Gosun' by Mr. Kuroda in the 1950s resulted in the 'Kuroda-Gosun' cultivar that has become grown widely, often simply referred to as 'Kuroda' (Figure 1). A range of

Asian and European carrots have been grown and adapted in Korea with most of the crop coming from fall planting until the 1970s when new varieties enabled cultivation in spring and summer. Most production consists of 'Kuroda', 'Danvers', or 'Chantenay' types (15-20 cm long), but some 'Nantes' types are also produced and "baby carrots" are also being tested for productivity and profitability in Korea. The fall crop historically consisted of open-pollinated cultivars but is rapidly being replaced by hybrids.

Korea and other Asian production areas use protected vinyl houses, tunnels, and overwinter cultivation methods to supplement summer and fall field harvests to supply carrots throughout the year. These practices raise a heightened breeding focus on early maturity, bolting resistance, and long storage life in addition to goals of *Alternaria dauci* resistance and smooth roots with uniform size, dark colour, and small cores, which all contribute to yield. New breeding efforts are underway to develop purple, red, and yellow carrots with added health benefits and new market niches.

Universities and research institutes have had carrot research underway since the 1980s. Hybrids currently account for most of the Korean and Japanese carrot production with future increases in the use of hybrids expected throughout Asia.

5.2 Incorporation of Cytoplasmic Male Sterility

Cytoplasmic male sterility (CMS) in carrot takes two basic forms: "brown anther" and "petaloid". The brown anther male sterility (Figure 2) was first discovered in the cultivar 'Tendersweet' and reported by Welch and Grimball in 1947. Brown anther sterility has also been found in several other cultivated (Banga et al., 1964; Michalik, 1971) and wild carrot sources. These plants begin forming normal anthers but development is halted as anthers fail to continue through to mature pollen production, remain rudimentary and turn brown. Brown anther sterility was used widely in the development of early carrot hybrids but it is frequently unstable (Michalik, 1979) so that in North American seed production areas and breeding materials, male fertile flowers often form on tertiary or secondary umbels. Brown anther sterility is used for most of the 'Nantes' hybrid seed produced in Europe and a stable brown anther has been developed for use in Korea with higher seed yield than petaloid types.

In 1953 the "petaloid" male sterility (Figure 2) was discovered in North American wild carrot by Munger (reported by Peterson and Simon, 1986). Petaloidy has also been found in other North American wild carrots (Morelock et al., 1996). Petaloidy is a homoeotic mutation where a second whorl of petals exists in place of anthers. Petaloid CMS is the most widely used form of male sterility for production of commercial carrot hybrids in North America today. It is stable across a wide range of environments throughout flowering and seed production, although in some genetic backgrounds petaloidy breaks down and late-season umbels can be fertile.

Several other forms and sources of cytoplasmic male sterility have been discovered and described in carrot (Ronfort et al., 1995). Between 1992 and 1996, three new CMS systems were developed from the cytoplasms of *D. carota* ssp. *gummifer*, *D. carota* ssp. *maritimus* and *D. carota* ssp. *gadecaei* (Nothnagel, 1992;

Steinborn et al., 1995; Linke et al., 1999; Nothnagel et al., 2000). Molecular analysis was performed in parallel and demonstrated large variability in the carrot mitochondrial genome, stable maternal inheritance of the mtDNA, and relatively large genetic distances between cultivated and wild relatives. Scheike (1992) and Scheike et al. (1992) found no correlation between mtDNA transcription and the expression of the male sterility. However, several recent reports point to a relationship between genome organization and transcription of the mitochondrial *atp9* (Szklarczyk, 1997; Szklarczyk et al., 2000), *orfB* (Nakajima et al. 2001) and *cox1* (Robison and Wolyn, 2006) loci in petaloid male-sterile carrots. An association between the cytoplasm and the ABC model of flowering based upon MADS-box genes was discovered by Linke et al. (2000; 2003).

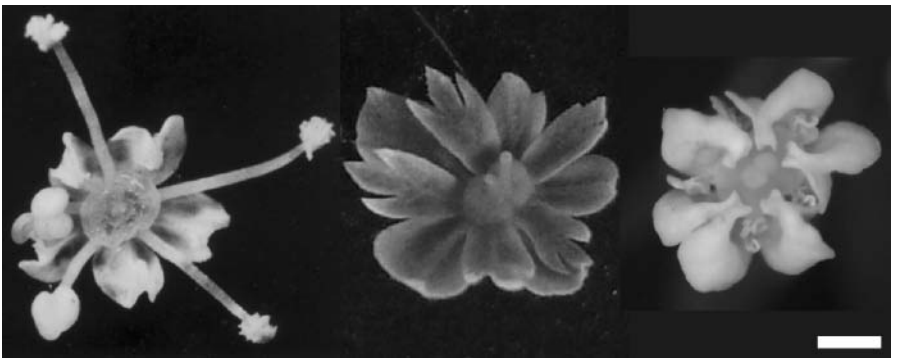

Fig. 2. Male fertile (left), petaloid male sterile (middle), and brown anther male sterile (right) carrot flowers. White bar = 1 mm.

The incorporation of CMS into carrot inbreds for the production of hybrid cultivars is similar to that for other crops. Generally this process begins at the F_2 or F_3 generation with the intercrossing of individual selected fertile plants to a male sterile plant. In the next generation progeny from the male sterile are examined for male fertility in the process of backcrossing for sterile line development. Presence of male fertile plants indicates the presence of restorer allele(s) from the original male fertile parent (Hansche and Gabelman, 1963). It has been relatively easy to identify and select male sterility maintaining carrot inbreds from a wide range of germplasm backgrounds but the incidence of restorers varies widely.

6 Carrot Breeding Goals

6.1 Production Traits

Production of a marketable crop is the top priority for carrot growers and consequently for carrot breeders. Adaptation to warm versus cool growing temperatures described earlier is a basic requirement to successful production that

receives much attention by breeders, but little is known about the genetics of temperature response in carrot. Cool season cultivars not only tolerate cooler temperatures, but they also tend to grow more slowly and resist premature flowering, while warm season cultivars tolerate heat better throughout their life cycle. Selection performed in various production environments has been successful in adapting the crop to a range of growing temperatures. As we better understand the genetic and physiological basis underlying carrot response to temperature, the breeding process can be more efficient.

The most critical trait that carrot breeders select against is tendency toward early flowering or bolting (Peterson and Simon, 1986). Early flowering is not tolerated in any commercial production since roots become fibrous and inedible even before the inflorescence is readily visible. While this trait alone does not distinguish temperate and subtropical carrots, the less frequent exposure of subtropical carrots to cool temperatures that induce bolting results in lower selection pressure to cool temperature-induced flowering. Consequently, a high incidence of early flowering is observed when subtropical carrot cultivars are grown in temperate production areas. The genetics of floral induction in carrot have not been described extensively, but a range of responses is often observed, so that combined with the unpredictability of the occurrence of cold weather in many climates, selection for this trait is an ongoing challenge for all carrot breeders.

Carrot breeders also direct attention to carrot haulms or tops. For some winter crop carrots, cool temperatures are a problem not only in inducing flowering, but also in inflicting frost damage to leaves and even roots. Consequently, selection for resistance to cold damage is an important trait in some breeding programs. Similarly, uniform seed germination under high soil temperatures and heat tolerance of larger plants are major goals pursued by breeders in subtropical production areas. The size and shape of carrot tops is also important since much of the commercial carrot harvest involves mechanically pulling roots from the soil by the tops. Excessive top size can be detrimental in forming a denser canopy conducive to the development of leaf blights and other diseases. The architecture of carrot tops varies widely across diverse germplasm and environments, providing breeders with a useful array of variation to work with. No reports of the genetic control of low or high temperature stress tolerance or of top size have been published.

Beyond these differences among carrot cultivars for incidence of premature bolting, adaptation to cooler and warmer climates, and top characteristics, differences in root shape are the major basis for distinguishing among carrots (Figure 1). At least 20 different root shapes have been described among European and Asian orange carrots with two “new” root types developed in the last century- ‘Imperator’ types in the U.S. in the 1920s and ‘Brasília’ in Brazil in the 1980s. Numerous temperate cultivar root shapes are grown today in both western and eastern production areas and their relative importance varies for differing markets. The predominant root shapes today for commercial production include ‘Nantes’ for Europe, ‘Kuroda’ for Asia, ‘Brasília’ for Brazil, and ‘Imperator’ for North America, but all of the other varietal groups (Figure 1) are also grown to some, sometimes substantial, extent. Intercrosses between root shape types can generate a very wide

range of progeny types and there may not be many genes involved in the control of this trait.

Uniformity of root shape and size are paramount requirements for growers and uniformity receives primary attention by breeders for all production regions (Peterson and Simon, 1986). The demand for storage root uniformity in length, diameter, and shape was a major motivation for developing hybrids. Quantitative evaluations of root shape and size are sometimes performed but most progress is achieved using visual selection. Since increased population density decreases root length, planting densities approximating that of the targeted market are utilized by breeders to make selections and test performance of the experimental cultivars.

Other important aspects of root appearance evaluated in breeding programs include root surface (lacking lateral roots, smooth and shiny), root integrity (freedom from cracking or splitting), crown or upper surface ("shoulder") of the root (no green colour and flat or slightly uplifted shape rather than concave or sunken), external colour (uniform orange), core size (small), and internal colour (uniform orange xylem and phloem) (Peterson and Simon, 1986; Rubatzky et al., 1999). Except for the major gene *Ck* for root cracking, the genetic control of these traits has not been reported, but visual selection based upon phenotype has been applied successfully in all production areas.

To improve open-pollinated populations or inbreds, the visual evaluation of root shape, uniformity and surface smoothness is initially made in the field. Most of the top is removed 2 to 5 cm above the crown and selected roots are stored for vernalisation. During vernalisation, roots are visually inspected and selected again before planting elite individuals to the field for seed production (Simon, 2000).

6.2 Disease Resistance

Disease and pests limit carrot production to some extent in all carrot production regions. Leaf blights caused by *Alternaria dauci*, *Cercospora carotae*, and *Xanthomonas campestris* pv. *carotae*, powdery mildew (*Erisiphe heraclei*), carrot fly (*Psila rosae*), cavity spot (*Pythium* species and perhaps other pathogens), and several nematodes (e.g. *Meloidogyne* spp., *Heterodera carotae*, *Pratylenchus* spp.) are among the most widespread carrot diseases and pests, occurring worldwide. Several other pathogens and pests can cause very serious damage in more limited regions (Rubatzky et al., 1999). Carrot breeders have relied upon natural infection in production areas where there is regular disease occurrence to make progress in selecting for genetic resistance for most diseases. Often highly susceptible cultivars or inbreds are interspersed among entries to be tested in the field and in some cases natural inoculation is supplemented with inoculum from artificially infested plants. This approach has been used in selecting for resistance to *Alternaria* leaf blight (Strandberg et al., 1972; Boiteux et al., 1993; Simon and Strandberg, 1998), carrot fly (Ellis and Hardman, 1981), and aster yellows (Gabelman et al., 1994). For soil borne disease and pests, heavily infested disease evaluation plots have been established for *Meloidogyne incognita, M. javanica* (Vieira et al., 2003), and *Pythium* sp. Methods for evaluating resistance to *Alternaria* leaf blight (Strandberg, 1988; Simon and Strandberg, 1998; Pawelec et al., 2006), cavity spot and

Rhizoctonia solani resistance (Breton et al., 2003), *M. hapla* (Wang and Goldman, 1996), and *M. javanica* (Huang, 1986; Simon et al., 2000) in controlled environments such as a greenhouse or growth chambers have also been developed.

Sources of varying levels of genetic resistance are known for all of these diseases (Simon, 2000) and selection for resistance has been initiated for *Alternaria* leaf blight (Boiteux et al., 1993; Simon and Strandberg, 1998; Vieira et al., 1991); *M. javanica* (Huang et al, 1986; Simon et al, 2000, Vieira et al., 2003), *M. incognita* (Vieira et al., 2003), *M. hapla* (Wang and Goldman, 1996); powdery mildew (Bonnet, 1983); carrot fly (Ellis and Hardman, 1981; Ellis et al., 1991), bacterial soft rot *Erwinia carotovara* (Michalik et al., 1992; 1997), and aster yellows (Gabelman et al., 1994) and the mode of inheritance has been described for resistance to all these diseases and pests except the latter two. Simply inherited resistance has been reported for *M. javanica* (*Mj–1*; Simon et al., 2000) and powdery mildew (*Eh*; Bonnet, 1983) with RAPD, AFLP, and SCAR markers developed for *Mj-1* to facilitate selection (Boiteux et al., 2000, 2004).

6.3 Consumer Quality

Selection for uniform orange colour has been exercised by carrot breeders for the last century. The nutritional quality conferred by the provitamin A carotenoids that account for the orange colour of carrots has received the attention of carrot breeders since the 1960s beginning with extensive efforts of W.H. Gabelman and his students (e.g. Laferriere and Gabelman, 1968; Umiel and Gabelman, 1972; Buishand and Gabelman, 1979). As a result, selection has raised provitamin carotene content in typical U.S. carrot varieties by 70% between 1970 and 1992 (Simon, 1992). Yellow, purple, red, and white carrots have received a renewed level of interest in recent years as growers look for new niche markets and consumers become more aware of the nutritional benefits of pigments. To support selection with objective measurements of colour, spectrophotometric evaluation tools have been developed (Baranska et al., 2005, 2006; Geoffriau et al., 2005; Surles et al., 2004).

Orange carrot colour is primarily due to alpha- and beta-carotene, yellow and red carrot colour are caused by carotenoids lutein and lycopene, respectively, and purple carrot colour is caused by anthocyanins (Umiel and Gabelman, 1972; Buishand and Gabelman, 1979; Simon and Wolff, 1987; Molldrem et al., 2004; Nicolle et al., 2004; Surles et al., 2004; Kurilich et al., 2005). When no pigments accumulate, carrots are white.

The commercial interest in carrots of unusual colours has stimulated research to determine the genetics underlying carrot colour. Genes for carotenoid accumulation described by Gabelman's group account for yellow and red colour classes (Buishand and Gabelman, 1979). Their efforts described seven major genes accounting for difference among orange, white, yellow, and red root colour. More recently the *Y* and Y_2 genes were mapped, a SCAR marker developed for Y_2 (Bradeen et al., 1997; Bradeen and Simon, 1998), and 20 QTL mapped for carotenoid content (Santos and Simon, 2002). A single major gene, P_1, confers purple storage root colour but this gene only accounts for part of the variation observed for purple colour, as a wide range of pigmentation patterns occur, and at least one other gene, P_2, influences

pigmentation in aerial plant parts (Simon, 1996). To develop breeding stock with potential commercial application, carrot breeders utilize traditional regional carrots and long-ignored heirloom cultivars with unusual colours in crosses with adapted, good-flavoured orange carrots to combine unusual colour with acceptable flavour for modern consumers.

Nitrates are important for their anti-nutritional value, especially for carrots used to make baby food. The inheritance of nitrate content in carrot is complex with incomplete dominance so that low-nitrate parents are necessary to obtain low–nitrate hybrids. In fact, while heterosis has significant positive effects upon many carrot production attributes, it is not observed for carotenoid or nutrient content, as midparent values are observed in the majority of hybrids. (Michalik et al., 1988).

Carrot flavour is a very important variable influencing consumer decisions. Flavour differences were noted between purple and yellow carrots hundreds of years ago and among modern orange carrot root types today, sweet and juicy flavour can be found in a wide range of types such as ‘Nantes’, ‘Kuroda’ and ‘Imperator’. With a broad genetic range in carrot flavour and the development of high value carrot products, including lightly processed “baby” or “cut and peel” carrots, improved raw carrot flavour has become a major breeding goal of carrot breeders in North America (Simon, 2000). Sweet flavour and succulent juicy texture are two of the major targets for improving raw carrot flavour. In addition to these two attributes, lack of harsh or turpentine flavour, caused by volatile terpenoids (Buttery et al., 1968), is the primary flavour component evaluated in selecting for improved flavour since high levels in harsh carrots can mask sweet flavour (Simon et al., 1980). Laboratory –facilitated selection is sometimes used for sweetness, using refractive index, colorimetric, or HPLC methods to quantify sugars; and for harsh flavour, using gas chromatography to quantify volatile terpenoids (Simon et al., 1982).

The genetics of raw carrot sweet and harsh flavour has been described and the patterns of inheritance are complex. Sweet flavour, not surprisingly, is associated with higher sugar content which is polygenic, although a single major gene, *Rs*, determines whether reducing sugars glucose and fructose, or sucrose, are the primary storage carbohydrates (Freeman and Simon, 1983; Stommel and Simon, 1989).

While texture is an important component of raw carrot flavour, little attention has been paid to the genetics of this trait. Since variation in texture interacts with perception of sweetness and harshness, breeder selection of carrot flavour generally relies upon tasting roots in the field and/or during the period they are being stored for vernalisation. Relatively little change occurs in carrot flavour or carotene content during early post harvest storage so it is a convenient time to evaluate quality attributes. Unfortunately, the brittleness that accompanies crisp texture tends to have a negative impact on the “durability” of carrots in mechanical harvesting and washing.

6.4 Yield

The attributes of carrot discussed above collectively account for carrot yield. Differing markets demand greater or lesser emphasis on specific attributes. For example, a much greater demand on appearance, size (not too small or too large),

and flavour is placed on carrots for fresh market sale than would be expected for forage carrots used as animal food. Total carrot yields can range from 30 to 100+ t/ha, but often a significant fraction can be discarded due to premature bolting, disease, improper size, shape, and colour. Consequently yield measurement in carrots, like all vegetable crops, focuses not on total yield, but rather marketable yield, or "pack-out". Carrot breeders focus their efforts on specific markets and then emphasize selection on those attributes important for those markets to improve net yield.

6.5 Seed Production

In addition to the grower and consumer needs, the breeder must develop breeding stock that can produce adequate economic seed yield. This aspect of carrot breeding has received little attention in terms of published research, but it is crucial to the success of a cultivar. For open-pollinated cultivars this is a relatively straightforward character to evaluate, but to produce hybrid carrot seed, several variables complicate breeding for seed yield. The male parent must produce adequate amounts of high quality pollen whereas the female parent must be able to produce adequate amounts of seed with high germination rate. The female parent must not get too tall in order to resist lodging. Furthermore, both parents should flower simultaneously and have a similar level of insect pollinator attractiveness. While their inheritance has not been studied, most of these traits can be effectively selected in the early phases of parental line development.

7 Breeding Methods and Techniques

7.1 Reproductive Biology

Carrot is an outcrossing, insect-pollinated diploid ($2n = 2x = 18$) species. It typically does not flower during the vegetative phase of its life cycle when the storage root forms and grows for 60 to 150 days (or more) depending upon environment and genotype. Early plant growth is slow as seedlings are established but then growth is rapid until interplant competition and seasonal climatic changes to suboptimal temperature limit growth (Rubatzky et al., 1999).

As a biennial crop, this vegetative phase of carrot life cycle is essential to successful crop production, but carrot breeders require flowering plants. Cool temperature is the primary stimulus that initiates carrot flowering so that plants exposed to cold weather in the field, or harvested and refrigerated, will make the transition from vegetative phase and initiate flowering. The amount of cold exposure necessary for this transition varies widely across diverse germplasm, with varieties developed for warmer climates generally requiring less cold before flowering begins. Carrots can be handled as an annual crop in breeding programs, typically raising a winter root crop in warmer production areas, harvesting and vernalising that crop at least 6 weeks in refrigerated storage, and then producing a summer seed crop in a cooler area to complete the cycle within one year.

Carrot flowers are protandrous and usually perfect, forming at most two seeds per flower. In addition to hermaphroditic flowers, there are also male-only flowers that occur at increased incidence with a rise in the order of umbels. A single carrot plant typically produces several hundred to several thousand flowers on multiple umbels over a few weeks (Rubatzky et al., 1999).

7.2 Inbreeding Depression and Hybrid Vigour

Self-pollination is not restricted by incompatibility and carrot inbreds developed for hybrid programs typically have included 2 to 5 generations of self-pollination in their pedigree. Like many outcrossing species, inbreeding depression can be severe in carrot but like most traits of interest to breeders, wide diversity in response to inbreeding is observed among cultivars and even from plant-to-plant within a population. Wild carrot also varies widely in response to inbreeding depression. Consequently, selection for resistance to inbreeding depression is effectively a major trait for carrot breeding programs where self-pollination is essential to breeding progress. Since the inception of hybrid breeding of carrots in the 1950s, breeders have developed hundreds of carrot inbreds in public and private carrot breeding programs. With this, inbred development now often begins with an intercross between two inbreds and because of this the occurrence and severity of inbreeding depression is generally reduced, relative to inbreds derived directly from carrots not previously exposed to inbreeding. This is not surprising as most deleterious recessive alleles would have already been eliminated in the development of the initial inbred parents.

Hybrid vigour is also quite variable in carrots (Michalik, 1979; Peterson and Simon, 1986; Rubatzky et al., 1999). The uniformity typical of hybrids that is so desirable for growers is usually very evident when two fairly inbred and unrelated inbreds are intercrossed, but less hybrid vigour and uniformity may be observed upon intercrossing less inbred individuals. Three way crosses using an F_1 single-cross female can also generate uniform and vigorous hybrids if the inbreds used to produce the female parent are carefully chosen.

7.3 Population Development and Improvement

Carrot breeders develop breeding populations that reflect the needs and interests of their production industry, but no serious effort has been exerted in establishing genetically diverse carrot breeding populations with broad-based, comprehensive input from a diverse range of breeders across production regions. Often the traditional open-pollinated cultivars of a particular region have served that purpose.

Population development and improvement requires starting populations with adequate heritable variation as well as methods for accurately measuring that variation. Population improvement for carrot has typically started with intercrosses among plants of open-pollinated varieties followed by phenotypic recurrent mass selection for root shape, smoothness, length, and colour useful in local production areas. When methods are available, selection has also been exercised for disease resistance and quality including resistance to *Alternaria* leaf blight, cavity spot,

nematodes; improvement of flavour, carotene content, and processing quality. During this process, low selection intensity is applied, focusing primarily on one or a few traits for a given population, although plants with major defects such as premature bolting, excessive root cracking and low plant vigour are always removed. When the trait or traits of interest come from cultivars not adapted to a targeted environment, for example incorporation of disease resistance from subtropical carrots into susceptible carrots being developed for temperate climates, the initial intercrosses need to be made between these two diverse groups, numerous F_1 hybrids intercrossed and selection made for both the "new" trait being brought in, as well as adaptation to the targeted environment. This approach has been used for introducing nematode resistance from subtropical gene sources into temperate germplasm, and for introducing high carotene content from temperate germplasm to subtropical cultivars (Simon, 2000). A similar approach was utilized to introgress cytoplasmic male sterility from wild to cultivated carrot (Peterson and Simon, 1986), although that process was made more complicated by the need to maintain and evaluate both sterile and maintainer stocks. The process of developing and improving broad-based populations in this way is sometimes referred to as pre-breeding and can serve as the starting point for both open-pollinated cultivars and inbreds.

7.4 Developing Open-Pollinated Cultivars

Open-pollinated carrot cultivars have been developed directly from other open-pollinated cultivars, from intercrosses between different open-pollinated cultivars, and from broad-based populations. Examples of each of these approaches include the development of 'Red-Cored Chantenay' from 'Chantenay,' 'Imperator' from a cross of 'Nantes' by 'Chantenay', and 'HCM' from diverse dark orange European and Asian cultivars, respectively (Simon et al., 1989). The process is similar to that described for population development and improvement above, but with more intense selection and testing.

Open pollinated and hybrid carrot breeding often include family selection and breeding (Figure 3), a recurrent selection strategy for trait improvement. Since selection occurs before plants enter the next sib-mating generation, like other biennial crops, pollen of plants failing to meet selection criteria never contributes to the subsequent generation.

7.5 Breeding Hybrid Cultivars

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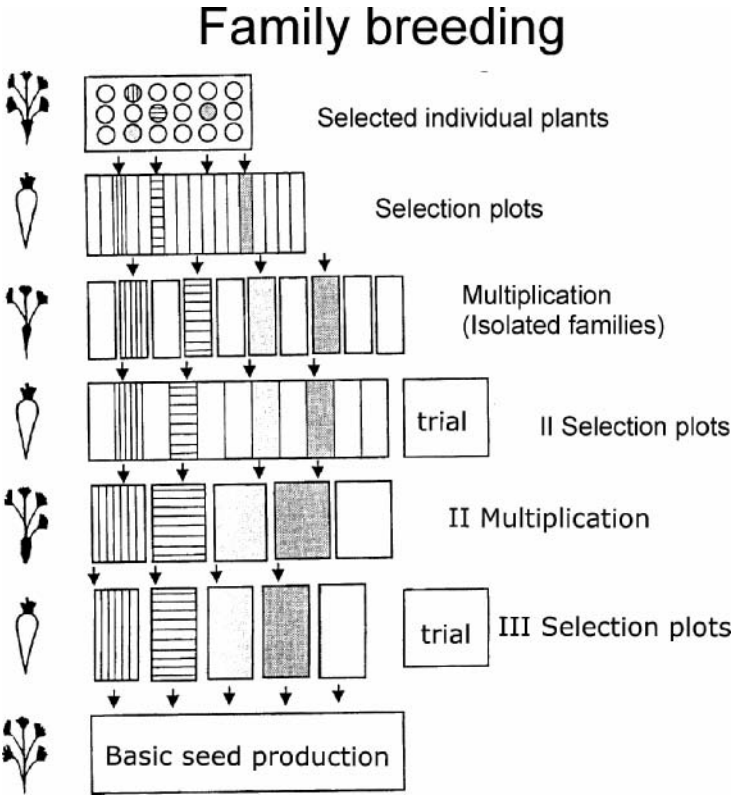

Fig. 3. Carrot family breeding over three cycles of selection. Plots with a flowering plant diagram to the left indicates the seed production phase of the cycle; those with a root to the left indicates the root production phase. Different shading patterns follow progeny of different plants selected in the first cycle.

8 Integration of Biotechnology in Breeding

8.1 Genetics

As is typical for many outcrossing, open-pollinated vegetable crops, relatively few genes have been described for carrot. A wide range of phenotypic diversity can be observed and not much of it has been genetically characterized. Approximately 50

genes have been associated with phenotypic traits including leaf, root, and floral characteristics (Simon, 2000; Nothnagel and Straka, 2003; Nothnagel et al., 2005). Genetics of carrot typically follows diploid inheritance patterns, as expected.

8.2 Genetic Markers and Genome Mapping

Before the 1990s few markers had been described for carrot and no linkages were reported. With the application of isozyme, and then DNA markers, several thousand genetic markers have been mapped for carrot (Schulz et al., 1994; Bradeen et al., 1997; Niemann et al., 1997; Westphal and Wricke, 1997; Vivek and Simon, 1999; Boiteux et al., 2000; Le Clerc et al., 2002; Just et al., 2007; Bradeen and Simon, 2007). Maps including at least 100 markers have been reported for F_2 populations, in some cases with backcrosses, but only one map-joining effort has been initiated (Santos and Simon, 2004). This will be an important effort in the near future. Most of the molecular markers to date have been dominant markers, mainly RAPDs and AFLPs. More codominant markers will be important for future mapping efforts.

8.3 Trait Mapping and Marker-Assisted Selection

Through the efforts of the Gabelman program in the 1960s – 1980s, the genetics of two multigenic traits of carrot were evaluated: carotenoids and restoration of CMS (Hansche and Gableman, 1963; Buishand and Gabelman, 1979). Several individual genes were identified with these efforts and the heritabilities of several carrot traits have been measured. Multigene trait mapping only began with the development of molecular markers and linkage maps. To date, seven monogenic traits have been mapped for carrot: *yel, cola, Rs, Mj–1, Y*, Y_2, and P_1. Work is underway to localize genes for flavour compounds and pigments, and to localize genes to specific chromosomes using fluorescent *in situ* methods. The inheritance of male sterility was described (Thompson, 1962), and the male fertile, petaloid, and brown anther mitochondrial genomes have also been characterized (Wolyn and Chahal, 1998; Bach et al., 2002; Linke et al., 2003). QTL have been mapped for carrot total carotenoids and five component carotenoids; phytoene, alpha-carotene, beta-carotene, zeta-carotene, and lycopene (Santos and Simon, 2002) and the majority of the structural genes of the carotenoid pathway is now placed into this map (Just et al., 2007). Marker-assisted selection has been reported for two genes: *Mj–1* (Boiteux et al., 2004) and *Rs* (Yau et al., 2005), with expanded selection for *Mj–1* expected in the future as this gene becomes more widely deployed and markers that are more unique for the resistance allele are developed.

8.4 Interspecific and Intersubspecific Crosses

An interspecific cross has been successful with *Daucus capillifolius* (McCollum, 1975), a source of genetic resistance to carrot fly (Ellis et al., 1991). Intersubspecific crosses with *D. carota* ssp. *gadecaei*, *D. carota* ssp. *azoricus, D. carota* ssp. *dentatus,* and *D. carota* ssp. *hispanicus* provide a broader range of variation in leaf,

root, and floral characteristics, and disease resistance (Nothnagel, 1992; Stein and Nothnagel, 1995; Steinborn et al., 1995; Linke et al., 1999; Nothnagel et al., 2000).

8.5 Tissue Culture, Transformation, and Protoplast Fusion

Carrot has been a model organism in the history of plant tissue culture. As one of the first plants used to demonstrate totipotency in the 1960s (Steward et al., 1964), carrot has been widely used for the study of plant regeneration. This technique has been used by seed companies to maintain and multiply elite parental lines of commercial hybrids. In addition to, and because of, its ease of regeneration, carrot transformation is quite efficient (Hardegger and Sturm, 1998; Baranski et al., 2006). Interesting carrot transformants have been developed for hepatitis B vaccine (Imani et al., 2002) and other unique plant products but like most other vegetables, agricultural deployment of transgenics has not been pursued, although herbicide and fungal resistance could have merit in crop production. Consumer acceptance of carrot transgenics could open that possibility but the widespread occurrence of wild carrot and consequent potential transgene escape could promote the development of specialized transgenic systems limiting this possible outcome.

Protoplast fusion involving carrot has been utilized to generate intergeneric fusion products with *Aegopodium* (Dudits et al., 1979), but the most extensive research involving carrot protoplasts has been the development of cytoplasmic hybrids or 'cybrids' fusing male fertile and petaloid CMS cytoplasms (Suenaga, 1991). Recovery of stable cybrids converting male fertile plants to male sterile, or vice versa, has not yet been realized and applied.

8.6 Haploids

The first carrot haploid reported came from a twin seedling and carrot haploids from anther culture have been reported by several projects (Anderson et al., 1990). A parthenogenetic approach has also been described recently. Some progress has been made in developing a system for obtaining carrot haploids with high frequency over a wide range of carrot diversity so that application of such a system in carrot breeding programs can be realized (Adamus and Michalik, 2003; Adamus et al., 2004).

8.7 Transposable Elements

The maize transposable elements *Ac* and *Ds* have been introduced into carrot and demonstrated transposition adequate for transposing tagging (van Sluys and Tempe, 1989; Ipek and Simon, 2006). This gene discovery technique could have application in carrot breeding and genetic studies. Several native transposable elements have also been identified in the carrot genome (Herrmann et al., 1988; Itoh et al.; 2002; Grzebelus et al., 2006) but transposition has not been observed. Immobilized members of these transposing families provide another molecular marker system for carrot genetic mapping.

9 Seed Production

9.1 Controlled Pollinations

Fully controlled pollination intercrossing one male fertile carrot plant with another requires emasculation and isolation of the seed parent followed by application of pollen with a small brush or stick. This is difficult and tedious as flowers are small (Figure 2) and only generate up to 2 seeds per pollination.

An easier alternative commonly used in Europe for intercrossing male fertile carrot plants takes advantage of protandry in carrots. A single, isolated umbel will not develop seeds even though pollen is present in the flowers. This umbel can serve as the seed parent in a cross if, one week after anthesis, the flowers of such an isolated umbel are sprinkled with water to flush out pollen. After it dries, pollen from the intended pollen parent can be introduced with a brush, and the seed parent umbel again placed in isolation. Seed produced are nearly always hybrid.

Another easy alternative that is commonly used is to isolate plants to be intercrossed in cloth or plastic screen pollination cages. Flies or bees are released in the pollinating cage to move pollen, or pollen is moved between plants by hand or brush. When a single pollen source is included in a cage, identity of paternal parent is obvious but when two male fertile plants are intercrossed in a pollinating cage both self-pollinations and crosses are possible and these two possibilities must be differentiated in the progeny by phenotypic or molecular markers, or by hybrid vigour when inbreds are intercrossed. Inbreds being evaluated for combining ability are typically isolated with several male sterile testers to allow evaluation of several hybrid combinations from a single pollinating cage (Peterson and Simon, 1986).

9.2 Root-to-Seed Production

All of the early generation seed production by carrot breeders utilize “root-to-seed” production whereby the root crop is grown to full or part season maturity, evaluated, vernalised and selected roots are then replanted in configurations suitable for covering with pollinating cages when flowering ensues. Root-to-seed production is used for all breeder’s seed and foundation seed, but it is also utilized quite widely for commercial carrot seed production and it provides breeders and seed producers an opportunity to select among plants to be included in the population or hybrid being produced. For seed production of plant populations that are too large to fit in a pollinating cage, variety integrity is ensured by geographic isolation of one seed lot from another by at least a few kilometres. It is also necessary to use production locations where no wild carrot occurs. Pollination is accomplished by providing honeybee hives, or in some cases, left to naturally occurring insect pollinators.

9.3 Root Storage and Vernalisation

The successful production of a root-to-seed carrot crop depends upon the production and storage of healthy roots capable of seed production. Roots are usually grown in a production area suitable for evaluation of traits important for the goals of the

breeding program. At harvest, most of the leaves are removed from 2-5 cm above the top of the root, and roots are usually placed into refrigerated storage (1°C to 5°C) for 1 to 7 months. Roots are usually selected for internal quality by excising the lower section of the root and visually examining xylem and phloem colour. The excised lower half can also be evaluated for flavour and/or laboratory analysis of pigments and sugars (Simon, 2000). Usually roots are packed in wood shavings or a similar absorbent of excess water, and placed in cold storage. In certain situations, selected roots are immediately replanted to the seed production areas, relying on cold climate to induce flowering. In some subtropical production areas, selection does not include cutting the root since this can greatly increase the incidence of root loss due to rot.

9.4 Seed-To-Seed Production

Much commercial carrot seed production utilizes "seed-to-seed" production whereby seed is planted in the mid-to late-summer growing season where the seed crop is to be produced, then plants are exposed to natural cold winter and the seed is produced the following year. While there is no need for root storage facilities with seed-to-seed production, the disadvantage of this system is that little selection can be applied and there is a greater risk for crop loss to severe winter conditions. Seed-to-seed production is sometimes used for 2 or 3 generations of self-pollination in early stages of inbred development if no selection for root characters is necessary. Pollination and variety integrity are managed as described for root-to-seed production.

9.5 Seed Production Diseases and Pests

The primary diseases of carrots during seed production are some of the same important ones during root production, namely *Alternaria dauci, A. radicina* and *Xanthomonas carotae*. All of these diseases can be seed-borne and contaminated seed may be a significant source of inoculum. The best control of bacterial blight is a hot water treatment of stock seed and variations of this measure to include bleach also reduces seed borne *Alternaria*.

Lygus bugs are a serious threat to carrot seed production. Control is challenging but essential to maintain high germination rate since their damage is very difficult to detect, hence eliminating the opportunity to remove damaged seed via conventional milling and cleaning methods. Red spider mites also threaten small-scale seed production. The chemical control of both of these pests usually injures insect pollinators as well, which further complicates seed production when they are present.

9.6 Seed Quality Control

After seed harvest of the foundation seed and commercial seed crops, quality control, including evaluation of germination rate and speed, and purity testing are performed. For the latter, isozyme or molecular markers specific for the two parental lines can be used. In addition to molecular procedures, the parental and hybrid crops are grown out and root type conformation is judged based upon breeder experience. This can be done in a winter root production site to allow evaluation as soon as possible.

10 Conclusions and Future Direction

Carrot breeding success has relied upon the availability of diverse germplasm to improve numerous traits. Expansion of carrot germplasm collections and systematic evaluation of them will be vital to future breeding success. Local carrot landraces that have been grown in isolated areas of Europe and Asia may soon become extinct as they are replaced by commercial cultivars. A more concerted effort to collect, maintain, and evaluate these potentially valuable materials should be initiated.

Carrot improvement has benefited greatly from the great breadth of diversity of the species so it may be surmised that carrot production could benefit from a more comprehensive international effort in developing populations focusing on resistance to particular diseases or improvement of particular production, postharvest, or consumer needs. Some possibilities may include *Alternaria* leaf blight, cavity spot, or root-knot nematode resistance; heat, cold, drought, or salinity tolerance; long-term cold storage; and texture, flavour or nutritional quality. To initiate such an effort, a collaborative system for evaluation of diverse germplasm will need to be established.

Carrot breeding has become much more focused and trait-driven in recent decades as the value of the crop has risen and new products have been developed. A prime example is the development of the "cut and peel" market for North America. Several traits that are critically important to growers and consumers, including root texture and resistance to cavity spot and *Alternaria* leaf blight, have not been well-described in terms of genetic control and combining ability. Techniques to reliably measure genetic variation for these and numerous other traits are essential for genetic models and breeding strategies to be developed. Marker-assisted selection has only recently been initiated in carrot. As the genetics of important complex traits become described, markers to more efficiently select them will warrant development. An international collaborative effort to establish robust genomic platforms (e.g. mapping populations, expression sequence tags, subtractive and large-insert libraries, allele mining, large-scale sequencing banks and expression arrays) for carrot and other Apiaceae would greatly facilitate the identification of molecular markers and discovery of genes associated with traits of breeding interest.

Carrot inbred and hybrid development has progressed to being reliable for a very broad range of cultivar types, and hybrids continue to rise in prominence. Progress is being made in the development of efficient methods to generate large numbers of carrot haploids from diverse germplasm and continued success in this endeavour could improve the process of inbred development dramatically.

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