HANDBOOK OF PLANT BREEDING

Jaime Prohens
Fernando Nuez
Editors

Vegetables II

Fabaceae, Liliaceae, Solanaceae, and Umbelliferae

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VEGETABLES II

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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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Family Umbelliferae (=Apiaceae)

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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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°N) to Northwestern Argentina (approx. 35°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°N to 32°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°C with germination inhibition in sensitive varieties at 30°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°C, more that 80% of the pollen produced was sterile. A high negative coefficient of correlation between temperature (15°C-35°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).

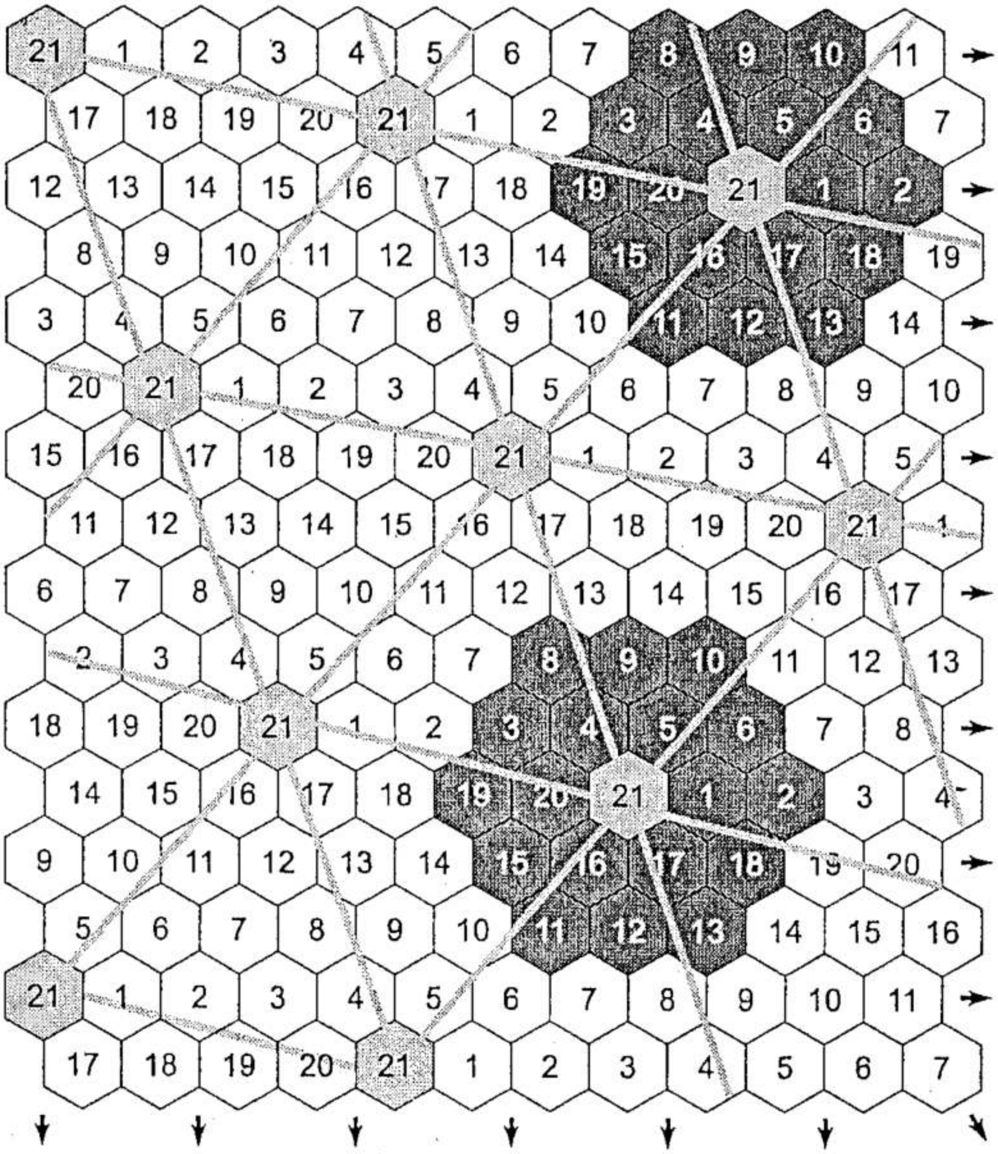

Fig. 1. The replicated R-21 honeycomb design evaluates a maximum of 21 entries. The moving replicate is exemplified by the darker shaded plots in the periphery of concentric circles whose central plot is entry 21 (Fasoulas and Fasoula, 1995).

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).

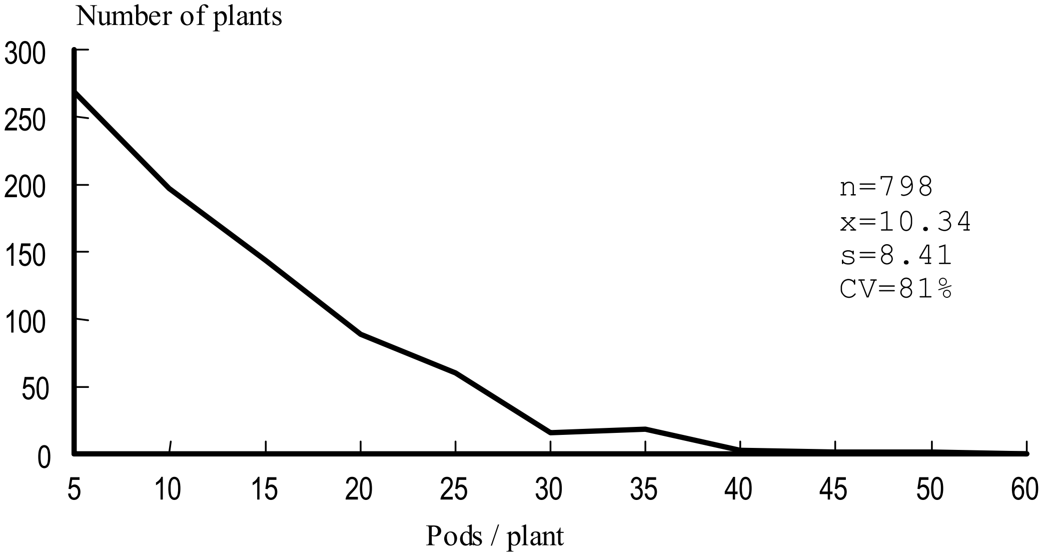

Fig. 2. Single-plant distribution of early maturing pods of source material 70 days after planting (Traka-Mavrona, et al., 2000).

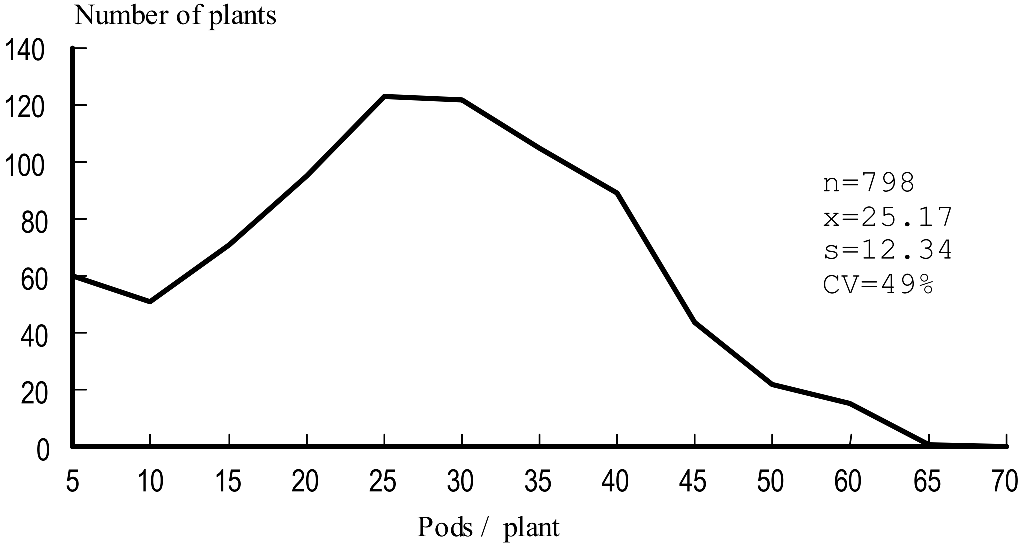

Fig. 3. Single-plant distribution of the number of pods of source material 78 days after planting (Traka-Mavrona et al., 2000).

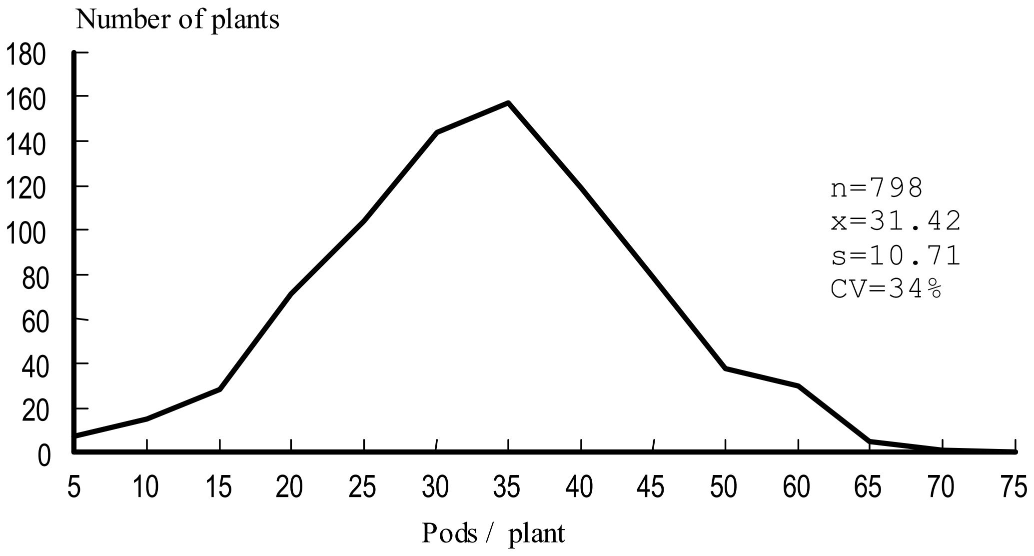

Fig. 4. Single-plant distribution of total number of pods of source material 92 days after planting (Traka-Mavrona et al., 2000).

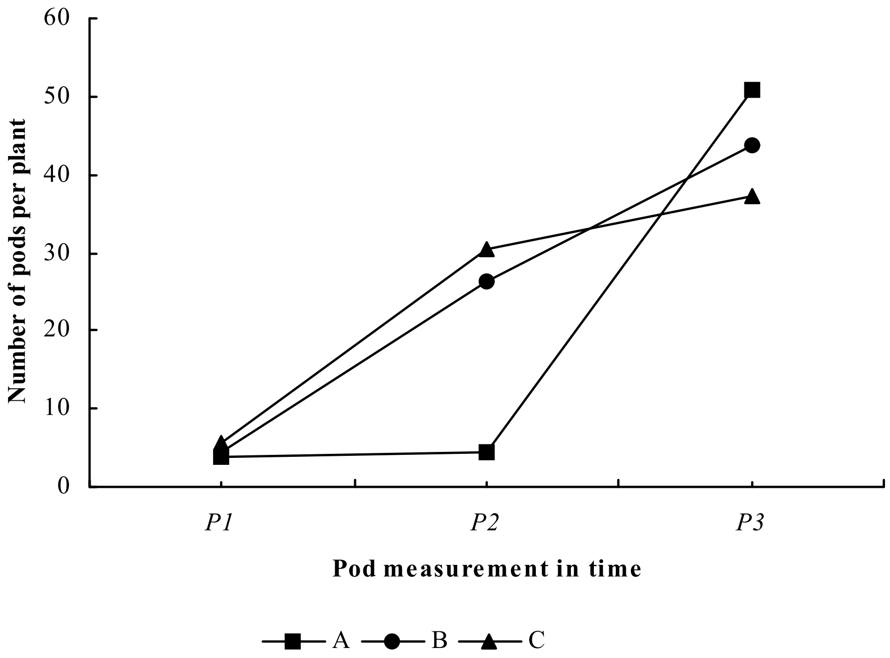

Fig. 5. Variation in the number of pods of each measurement in time of the source variety as affected by the planting date. A, B, C = the three planting dates. P1, P2, P3 = the three pod measurements (Traka-Mavrona et al., 2002b).

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).

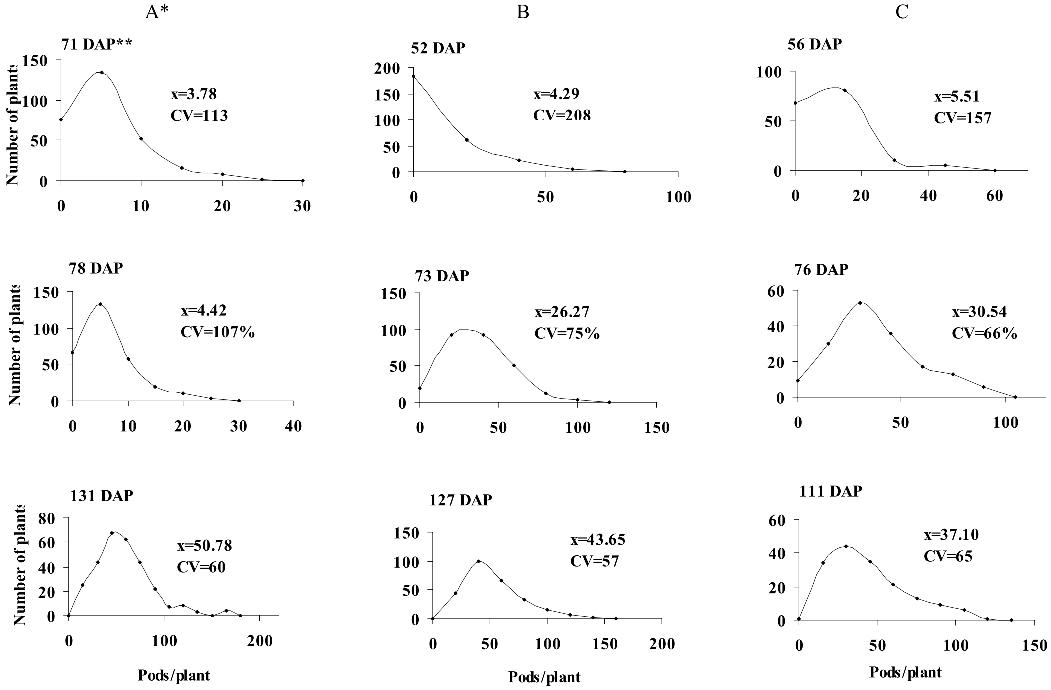

Fig. 6. Single-plant frequency distributions of first (upper), second (centre) and third (below) pod measurement of the source variety at each of the growing environments (planting dates). *A (left), *B (centre), *C (right) = the three planting dates, **DAP = days after planting (Traka-Mavrona et al.,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