8

Pigeonpea

Kiran K. Sharma[1], Gopinath Sreelatha[1,2], P. Bhatnagar-Mathur[1], Kul Bhushan Saxena[1] and Daggu M. Rao[2]

[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, [2]Department of Genetics Osmania University, Hyderabad, Andhra Pradesh, India

1. INTRODUCTION

Pigeonpea is one of the major grain legume crops grown in the tropics and subtropics. It is usually grown in rainfed areas that are prone to drought. Because of its high protein content, pigeonpea forms a significant component of the diet of the vegetarians in the semi-arid tropics (SAT). Genetic improvement of pigeonpea has received considerable attention over the years from plant breeders with the aim of increasing the grain yield and to minimize crop losses due to unfavorable environmental conditions, and attack by various pests and pathogens. Conventional plant breeding coupled with improved farm management practices have led to a significant increase in world pigeonpea production. Conventional breeding of pigeonpea has succeeded in producing short duration varieties and better cultivars with yield improvement, which are being grown in different parts of the world. Pigeonpea shows considerable yield losses due to various biotic and abiotic constraints. Though the wild accessions of pigeonpea are rich source of resistance to these constraints, the introgression of genes conferring resistance or tolerance to these stresses into cultivars is difficult due to cross-incompatibility. Linkage drag of desirable genes with undesirable genes also complicates such breeding programs. Biotechnological approaches, such as gene transfer for enhanced disease and pest resistance offer opportunities for rapid improvement of pigeonpea. In recent years, biotechnology has emerged as one of the important tools for agricultural research. In concert with traditional plant breeding practices, biotechnology is contributing toward the development of novel methods to genetically alter and control plant development, plant performance, and plant products. Genetic engineering offers a possible solution by lowering the farm level production costs through making plants resistant to various abiotic and biotic stresses and by enhancing the product quality (i.e., by increasing the appearance of end product, nutritional content, or processing or storage characteristics). Therefore, pigeonpea improvement efforts have focused on raising the yield potential, quality characteristics, and resistance to biotic and abiotic stresses depending on the regional requirements of the crop through biotechnological approaches. This review deals with the recent advancements in pigeonpea breeding and emerging transgenic innovations that would play a significant role in the future pigeonpea improvement programs and offer many new opportunities to develop it as a new generation legume food crop.

Compendium of Transgenic Crop Plants: Transgenic Legume Grains and Forages. Edited by Chittaranjan Kole and Timothy C. Hall
 ISBN 978-1-405-16924-0

1.1 History, Origin, and Distribution

There has been a major dispute on the possible origin of pigeonpea. Several conclusions have been made in favor of India given the presence of several wild relatives, the large diversity of the gene pool, ample linguistic evidences, a few archaeological remains, and the wide usage in daily cuisine (Van der Maesen, 1983). Pigenpea now acclimatized in several tropical countries was cultivated in ancient Egypt, Africa, and Asia since prehistoric times. The name pigeonpea was first reported from plants used in Barbados where it was used as pigeon feed which led to the name "pigeonpea" in 1692 (Van der Maesen, 1986). The major producer of pigeonpea is India with over 100 cultivars, 2.4 million hectares cultivated and 90% of world production.

1.2 Botanical Description

1.2.1 Taxonomy

Pigeonpea, *Cajanus cajan* (L.) Millsp., belongs to the subtribe Cajaninae of the agriculturally most important tribe Phaseoleae under the subfamily Papilionoideae of the family Leguminosae and is the only cultivated food crop of the Cajaninae subtribe (Purseglove, 1988). Lackey reviewed the Phaseoleae as a group and realigned Bentham's classical classification (Bentham, 1837; Bentham and Hooker, 1865) taking into account the genera described since the last century. The tribe Phaseoleae also includes many important crops such as soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris* L.), and mungbean (*Vigna radiata* L. Wilczek) (Young *et al.*, 2003). Pigeonpea has a diploid genome comprising 11 pairs of chromosomes ($2n = 22$) (Greilhuber and Obermayer, 1998). Karyotype analysis of both mitotic and meiotic chromosomes of pigeonpea, various wild species, and their interspecific hybrids indicated high homology and almost complete meiotic pairing.

Among the members of Phaseoleae, Cajaninae is well distinguished by the presence of vesicular glands on the leaves, calyx, and pods. Currently, 11 genera remain under Cajaninae, including *Rhynchosia* Lour, *Eriosema* DC., G. Don, *Dunbaria*, W. & A. and *Flemingia* Roxb. ex Aiton. The members of the earlier genus *Atylosia* closely resemble the genus *Cajanus* in vegetative and reproductive characters. However, they were relegated to two separate genera mainly on the basis of the presence or absence of a seed strophiole. In 1986, Van der Maesen revised the taxonomy of *Cajanus* and merged the two genera under *Cajanus* following systematic analysis of morphological, cytological, and chemotaxonomical data, which indicated the congenicity of the two genera. *Cajanus* is now recognized as having 32 species most of which are found in India and Australia. Wild *Cajanus* species exist mostly as remnants of cultivations and persist in forests in several places. The closest wild relative, *Atylosia cajanifolia* Haines, has been found in some localities in East India and most other *Atylosias* are found scattered throughout India. In Africa, *Cajanus kerstingii* grows in the drier belts of Senegal, Ghana, Togo, and Nigeria. Pigeonpeas occur throughout the tropical and subtropical regions, as well as the warmer temperate regions (as North Carolina) from 30 °N to 30 °S (Duke, 1981).

Pigeonpea is divided into two botanical varieties "var. *flavus*" and "var. *bicolor*". The cultivars of var. *flavus* are earlier maturing, have shorter plants with yellow standards, and green glabrous pods, which are light colored when ripe, and are usually three seeded. These are the *tur* cultivars of India, where they are extensively cultivated in the Peninsula. The cultivars of var. *bicolor* are perennial, late maturing, large, bushy plants, with dorsal side of standard red or purple or streaked with these colors, and hairy pods blotched with maroon or dark colored, with 4–5 seeds, which are darker colored or speckled when ripe. These are the *arhar* cultivars of India, which are more extensively cultivated in the north of the country (Purseglove, 1988).

1.2.2 Plant habit and morphology

Among the grain legumes only the pigeonpea has not been subjected to a radical change in life form. Pigeonpea is an erect perennial legume shrub often grown as an annual, attaining height of up to 5 m. Pigeonpea is almost exclusively grown as an annual, in rows and/or mixed with any other crops such as cotton, sorghum, millets, and groundnut, which are harvested several months

prior to pigeonpea. The pigeonpea plants are cut down when most of their pods have ripened, often when green leaves are still present. Spreading forms are preferred for filling the gaps formed by earlier harvested intercrop. Erect cultivars may be useful for intercropping with other crop species of similar duration, but have not proved better than spreading ones.

Pigeonpea leaves are trifoliate, green, and pubescent above and silvery grayish-green with longer hairs on the underside and spirally arranged on the stem. While the pigeonpea seedlings emerge 2–3 weeks after sowing (Duke, 1981), the vegetative growth begins slowly and accelerates at 2–3 months. Flowers occur in terminal or axillary racemes and are 2–3 cm long (Purseglove, 1968), usually yellow, but can be flocked or streaked with purple or red. Pods are flat, usually green in color, sometimes hairy, sometimes streaked, or colored dark purple, with 2–9 seeds/pod. Seeds are, widely variable in color, 6–9 mm in diameter, and weigh 4–25 g/100 seed (Sheldrake, 1984). Roots are thin with a deep-rooting taproot reaching up to 6 ft. (2 m) in depth. This deep-rooting system helps to improve water infiltration into the soil.

1.2.3 Climatic and soil requirements

Pigeonpea needs moist and warm weather during germination (30–35 °C), 20–25 °C during active vegetative growth, and about 15–18 °C during flowering and pod setting; however, at maturity it needs higher temperature of around 35–40 °C. Waterlogging, heavy rains, frost are very harmful for the crop (Chauhan, 1987). Hailstorm or rain at maturity damages the entire crop. The crop may be grown on any type of soil but sandy-loam to clayey-loam soils are supposed to be best. Soil must be very deep, well drained, and free from soluble salts.

1.2.4 Ecology

Pigeonpea is hardy, widely adaptable, and more tolerant of drought and high temperatures than most other crops. It grows best at a soil pH of 5.0–7.0, but tolerates a wider range (4.5–8.4). It grows on acid sands in the Sahel and alkali clays in India. Ranging from warm temperate moist to wet through tropical desert to wet forest life zones, pigeonpea has been reported to tolerate annual precipitation of 5.3–40.3 dm (mean of 60 cases 14.5 dm), annual mean temperature of 15.8–27.8 °C (mean of 60 cases = 24.4 °C), and pH of 4.5–8.4 (Duke, 1981). The traditional varieties of pigeonpeas grown by farmers in India in the early 1970s were photoperiod sensitive medium- to long-season types.

1.3 Economic Importance

Pigeonpea plays an important role in food security, balanced diet, and alleviation of poverty because it can be used in diverse ways. Pigeonpea seed contains 20–30% protein, is rich in essential amino acids, carbohydrates, and minerals (Faris *et al.*, 1987; Saxena *et al.*, 2002), and is the principal source of dietary protein for an estimated 1.1 billion people, most of whom are vegetarian and poor (Jones *et al.*, 2004). Pigeonpea contains approximately 57.3% carbohydrates in dried seed. The protein and carbohydrate composition of Indian split dal is 22.3% and 57.2%, respectively (Purseglove, 1988). The crop can be described as unique because it is a legume and a woody shrub. Pigeonpea is grown for its seed for human consumption and for income generation by trading surpluses in local and commercial markets. Besides its main use as dal (dry, dehulled, split seed used for cooking), the green seeds are cooked as a vegetable in Africa, Central America, and the states of Gujarat and Karnataka in India, tender pods are cooked whole in Brazil, Thailand, and the eastern islands of Indonesia. Green peas are processed for canning and freezing in Central America and India for export to North America. The seed husks, pod walls, and green leaves are commonly fed to cattle. Branches and stems can be used for making baskets and as fuel. Pigeonpea is also used as a shade crop and wind break (young coffee, forest seedlings), cover crop, or as support (vanilla) (Duke, 1981). It has an inherent ability to withstand environmental stresses (especially drought) due to the fast growing, deep, extensive root system making it one of the most sought after crops in plant introduction trials aimed at bringing new areas under cultivation (Okiror, 1986). The slow growth of the plant above ground during

its early phase offers very little competition to other crops and allows productive intercropping with virtually any crop. It is grown as a sole crop or as an intercrop mixed with cereals (maize, sorghum, pearl millet, finger millet), fiber, and other legume crops (groundnut, soybean) under wide climatic conditions in rainfed low-input agricultural systems. Pigeonpea adapts to different climates and soils except those that are excessively wet or experience frost (Troedson *et al.*, 1990). The effect of pigeonpea on soil fertility has been studied in detail (Ong and Daniel, 1990). It contributes to the C, N, and P economy of the soil (Kumar Rao *et al.*, 1987; Rego and Nageswara Rao, 2000; Fujita *et al.*, 2004), enhancing its performance even under marginal input. Pigeonpea is tolerant to low P supply and acid soils as well as having a high capacity for incorporation of external P into organic P (Fujita *et al.*, 2004). Its critical requirement of P concentration for dry matter production is low compared to other major protein crops like soybean (Adu-Gyamfi *et al.*, 1990). This benefits both the pigeonpea crop and subsequent crops in rotation, thus contributing to increased productivity and soil amelioration (Duke, 1981; Ae *et al.*, 1990). Pigeonpea is also used to restore soil fertility and to prevent soil erosion (Al-Nahidi *et al.*, 2001; Xuxiao *et al.*, 2002). Morton (1976) listed many folk medicinal uses of pigeonepa. Dry roots, leaves, flowers, and seeds are used in different countries to treat a wide range of ailments of the skin, liver, lungs, and kidney. Because pigeonpea is a low-input rainfed crop with characteristics that provide economic returns from each and every part of the plant, its cultivation has a direct bearing on the overall economic and financial well being, and on the nutritional status of subsistence farmers in the Asian subcontinent.

A good crop of 1ha gives about 25–30 quintals of grains and about 50–60 quintals of sticks and 10 quintals of dried leaves in the form of straw. In India, pigeonpea is mainly cultivated by small and marginal farmers, accounting for 85–90% of the world's area under pigeonpea cultivation. Although, in India, there has been a considerable increase in the area under pigeonpea cultivation from 2.18 to 3.82 m ha^{-1}, and the production from 1.72 to 2.88 million tons between 1950–1951 and 1996–1997, there was a significant drop in productivity from 780 to 753 kg ha^{-1} during the same period (AICPIP, 1999). Andhra Pradesh accounts for 10.2% of area and 4.26% of the pigeonpea production in the country. Globally, pigeonpea has recorded a 43% increase in area since 1970. It is currently grown on 4.3 m ha^{-1}. India is the largest producer with 3.2 m ha, followed by Myanmar (580 000 ha), China (60 000 ha), and Nepal (28 000 ha). In Asia, between 1972 and 2003, pigeonpea recorded 57% increase in area (2.44–3.81 m ha^{-1}) and 61% increase in production (1.72 to 2.77 million tons). In Africa between 1972 and 2003, pigeonpea recorded 66% increase in area (0.26–0.42 m ha^{-1}) and 96% increase in production (0.13 to 0.26 million tons) (http://www.icrisat.org/PigeonPea/PigeonPea.htm). Pigeonpea is now reported to be grown in 50 countries of Asia, Africa, and the Carribbean. The current global annual production of pigeonpea is valued at more than US$ 1700 m (FAOSTAT, 2005).

1.4 Constraints to Pigeonpea Productivity

The production of pigeonpea has remained static over the last several years (Souframanien *et al.*, 2003). The yield on farmers' fields is low due to a number of factors. A large variation is seen in productivity across years as farmers continue to grow their traditional landraces that frequently suffer from several biotic and abiotic stresses due to lack of quality seed. Poor production practices such as low plant densities, low soil fertility, insufficient weeding, and insufficient/inappropriate use of fungicides and herbicides are other constraints. Environmental (frequent droughts, easily erodible soils with poor waterholding capacity) and socioeconomic (lack of roads, marketing infrastructure, and exploitation by middlemen) factors also affect productivity. Apart from these, biotic and abiotic are the most important constraints for pigeonpea production and are listed below.

1.4.1 Biotic factors

Biotic stresses due to fungus, bacterial, and viral diseases, and insects pests cause heavy losses in yield of pigeonpea. Fungal diseases involving 45 pathogens are known in pigeonpea and the most important and widespread disease is wilt (*Fusarium udum*), favored by soil temperatures

of 17–20 °C. It affects the plants at all stages of its development and in India it causes a loss of 5–20% and in severe case upto 50% loss is observed. *Fusarium* wilt is especially prevalent in India and East Africa, where field losses of over 50% are common (Marley and Hillocks, 1996). Other fungi include: *Cercospora indica* (leaf spot), *Colletotrichum cajanae, Corticium solani, Diploidia cajani* (stem canker), *Leveillula taurica, Macrophomina phaseoli, Phaeolus manihotis, Phoma cajani, Phyllosticta cajani, Phytophthora cajani* (stem blight), *Rhizoctonia bataticola, Rosellinia* spp., *Sclerotium rolfsii,* and *Uredo cajani* (rust). So far, economic damages by these have been small or negligible, but rust is locally of some importance. Pigeonpea is also attacked by the bacterium *Xanthomonas cajani* that causes leaf spot and stem canker diseases (Kay, 1979). The sterility mosaic disease caused by sterility mosaic virus is being recognized as a serious economic threat as it can cause complete crop failure (Kay, 1979).

Nematodes that are of minor importance are Helicotylenchus cavevessi, H. dihystera, H. microcephalus, H. pseudorobustus, Heterodera spp., H. cajani, H. trifolii, Hoplolaimus indicus, Meloidogyne hapla, M. incognita acrita, M. javanica, M. javanica bauruensi, Pratylenchus spp., Radopholus similis, Rotylenchulus reniformis, Scutellonema bradys, Scutellonema clathricaudatum, Trichodorus mirzai, Tylenchorhynchus brassicae, T. indicus, Xiphinema campinense, and X. ifacolum. Damage caused by insect pests is a major constraint on yield in most areas. The podborer, Helicoverpa armigera, is commonly regarded as the key pest throughout Africa and Asia. It is particularly damaging on early formed pods. In many parts of India, the podfly, Melanagromyza obtuse, takes over as the dominant pest later in the season. In some areas, a newly recognized hymenopteran pest, Tanaostigmodes, can also cause extensive pod damage late in the season. Pests, which can be locally or seasonally important, are plume moth (Exelastis atomosa), blue butterfly (Euchrysops cnejus), leaf tier (Eucosma critica), bud weevil (Ceutorhynchus aspurulus), spotted pod borer (Maruca testulalis), pea pod borer (Etiella zinckenella), and bugs (Clavigralla spp.). A blister beetle (Mylabris pustulata), which destroys flowers, can be a spectacular but localized pest (Kay, 1979). Thrips (Frankliniella schultzei, Megalurothrips usitatus) may cause premature flower drop. In general, the determinate (clustering) plants lose more to lepidopterous borers while podfly causes more damage to the later indeterminate cultivars.

1.4.2 Abiotic stresses

Drought, cold heat, and salinity are the abiotic stresses that affect the pigeonpea yield. In India pigeonpea is grown predominantly in the states of Uttar Pradesh, Gujarat, and Maharashtra, which together contribute about 85% of the total growing area and production of India (Muller *et al.*, 1990). More than 51% of the saline soils in India are located in these states (Agarwal *et al.*, 1976). Among cultivated legumes, pigeonpea is classified as moderately sensitive to salinity (Keating and Fisher, 1985). Waterlogging, heavy rains, frost are very harmful for the crop. Hailstorm or rain at maturity damages the entire crop.

1.5 Pigeonpea Breeding

Many traditional breeding tools including selection, hybridization, mutation, and polyploidy, have been employed in pigeonpea improvement and some of them have been successful in improving the crop. Development of extra-early varieties and resistance to drought and waterlogging were identified as important breeding targets. Pigeonpea produced by resource-poor farmers is more vulnerable to attack by disease and insect pests and to abiotic stresses. To combat these stresses usage of pesticides, fertilizers, and irrigation are a common practice. Utilization of such inputs, however, can seriously reduce profitability and threaten the environment, and many pests are not effectively controlled with chemicals. Thus, across farming systems, biotic and abiotic stresses continue to represent the major constraints on subsistence production and economic yield of pigeonpea. Development of cultivars with improved resistance to biotic and abiotic stresses is a primary goal of pigeonpea breeding programs throughout the world. Some of the conventional breeding methods employed in pigeonpea improvement are delineated below.

1.5.1 Wide hybridization

In any crop improvement program, the parents used in the hybridization are generally different varieties of the same species. Hybrids produced from intervarietal crosses possess maximum fitness value and are favored under both nature and domestication. But, in many cases, it may be desirable or even necessary to cross individuals belonging to two different species or genera. Wide crosses or more precisely interspecific and intergeneric hybrids do not occur naturally and are eliminated by the natural forces because of nonviability and/or sterility of the hybrids. In certain crops, plant breeders in the 20th centaury have increasingly used interspecific hybridization for transfer of genes from a noncultivated plant species to a crop variety in related species. Distant hybridization is mostly aimed at introducing new genetic variability or to achieve a new genomic constitution in such a way that the characters of the parental species are recombined effectively. These possibilities are directly related to the degree of genetic relatedness between the parents, i.e., the closer the genome relationship between the cultivated and the wild species the greater the amount of genetic recombination, and consequently variability. Valuable characters present in wild species that can be utilized in improvement of pigeonpea cultivars and the attempts made to transfer them to cultivars are reviewed hereunder.

Interspecific hybridization in *Cajanus* species dates back to 1956 when Deodikar and Thakur (1956) made the first cross between *C. cajan* with *C. lineatus* and obtained fietile hybrids. Roy and De (1967) obtained a hybrid between *C. cajan* and *C. scarabeoides.* The prospects of potential gene transfer for pod borer resistance, drought resistance, high fruit set, higher seed protein content, early maturity, etc., from some *Atylosia* and *Cajanus* species have been observed (Pundir and Singh, 1987).

Pundir and Singh (1987) obtained fertile hybrid from the crossings of *Atvlosia cajanifolius* with the cultivated varieties, Pant A2 and UPAS 120. Considering the value of the traits, namely, leaf spot resistance, pod fly resistance, seed size, and high methionine content possessed by *Cajanus cajnaifolius*, crosses involving this species might throw useful segregants in adavanced generations. Similarly, *Cajanus scarabaeoides* another species of the secondary gene pool, tolerates drought and exhibits mechanical resistance and antibiosis to *Helicoverpa* in its pods. This species can easily be crossed with pigeonpea. The high protein content (28.3%) in some accessions of this species has been transferred to pigeonpea (Saxena *et al.*, 1997). Studies carried out by Dodia *et al.* (1996) revealed that larval and pupal mass of *Helicoverpa* fed on wild pigeonpea flowers and F_1 hybrids of *C. scarabaeoides* and cultivated *C. cajan* were significantly lower than those for larvae fed on the cultivated pigeonpea indicating the scope of transferring of resistance from this wild species to the cultivated pigeonpea. Unfortunately, there has not been much success in crosses between cultivated *C. cajan* and *Cajanus sericeus*. *C. sericeus* is known for high fruit set, 5–6 seeds per pod, drought tolerance, pod borer resistance, and high seed protein content. Ariyanayagam *et al.* (1993) noted poor success when *C. sericeus* was taken as maternal parent of crosses with pigeonpea as it produced male sterile lines having mitochondria of the *C. sericens*. Another species of secondary gene pool having salinity tolerance is *Cajanus albicans* (Subbarao, 1988). Kumar *et al.* (1985) obtained 7% success, when *C. cajan* was taken as a female in crosses with *C. albicans*. Whereas, Ariyanayagam *et al.* (1993) noted that crosses of *C. albicans* with pigeonpea with latter as male parent resulted in high level of male sterility in F_1 generation (Table 1).

Cajanus acutifolius, a species of Australian native, belongs to the secondary gene pool. It is drought tolerant and crossable with pigeonpea. Fertlie seeds were not formed in hybrids when *C. acutifolius* was used as female parent (Ariyanayagam *et al.*, 1993). *Cajanus platycarpus* is the only species of tertiary gene pool that has a great potential donor traits including resistance to *Fusarium* wilt (*F. udum*) and *Phytophtara* blight (*Phytophthora drechsleri* f. sp. *cajani*) diseases, high pod set and large seed size (Pundir and Singh, 1987), and salinity tolerance (Subbarao, 1988). Ariyanayagam and Spence (1978) reported successful hybrids between *Atvlosia platycarpus* and pigeonpea, and suggested that genes for earliness and insensitivity to day length could be transferred from *A. platycarpus* in to pigeonpea.

Table 1 Wide hybridization and important traits in wild species of pigeonpea

Wild species	Useful characters	Remarks	References
C. scaraboides	Drought tolerant, mechanical resistance, and antibiosis to Helicoverpa, high protein content (28.3%)	High protein content transferred to pigeonpea, larval and pupal mass reduced when fed on F_1 hybrids	Dodia *et al.*, 1996
C. sericeus	High fruit set, 5–6 seeds/pod, drought resistant, pod borer resistant, high protein content (28.6%)	Male sterile hybrids with *C. sericius* as maternal parent	Ariyanayagam *et al.*, 1978; Wanjari, 1998
C. albicans	Sterility mosaic resistant, high seed protein content (28.7%), salinity tolerance	7% success in crosses with *C. cajan* as female parent, male sterility in F_1 hybrids with *C. cajanus* as male parent	Kumar *et al.*, 1985; Subbarao, 1988; Ariyanayagam *et al.*, 1978
C. acutifolius	Drought tolerant, sterility mosaic resistant, high seed protein content (28.7%)	Male sterile F_1 hybrids with both *C. cajanus* as female and male parent	Ariyanayagam *et al.*, 1978
C. platycarpus	Resistant to *Fusarium* wilt and *Phytophthora* blight, early maturity and annuality, high pod set and large see size, high protein content (29.3%) salinity tolerance	F_1 hybrids were produced, F_1 hybrid with under developed seeds, reciprocal pollination using *C. platycarpus* as male parent was unsuccessful	Ariyanayagam and Spence, 1978; Saxena *et al.*, 1996
C. cajanifolius	Leaf spot and pod fly resistant, high methionine content, high protein content (29.2%)	Fertile F_1 hybrids, semi-fertile F_1 hybrids with forage potential, high protein content in F_1 hybrids	Pundir and Singh, 1987

1.5.2 Heterosis breeding

Heterosis is the superiority in the performance of hybrid over both the parents. Commercial exploitation of heterosis in crop plants is regarded as a major breakthrough in the realm of plant breeding. It has lead to considerable yield improvement of several cereals and other crops (Rai, 1979). Exploitation of "hybrid vigor" or "heterosis" showed a quantum jump in yields in some cereals and vegetable crops in the past. In legumes, this system could not be exploited for enhancing the productivity, primarily due to their cleistogamous nature of flowers that do not permit economical mass pollen transfer, necessary for large-scale hybrid seed production (Saxena *et al.*, 1997). Pigeonpea, however, is an exception where insect-mediated natural outcrossing up to 70% has been reported (Saxena *et al.*, 1990). Solomon *et al.* (1957) were the first to report hybrid vigor in pigeonpea for yield as 24.5%. The availability of cytoplasmic male sterility (CMS) in this crop (Tikka *et al.*, 1997; Wanjari *et al.*, 2001; Saxena and Kumar, 2003) has opened up the possibilities of developing commercial hybrids. The discovery of stable male sterility systems, availability of natural outcrossing, and evidence of yield advantage has set a perfect stage for increasing yield through developing high yielding widely adapted hybrids to break the persisting yield plateau in pigeonpea.

Male sterility is the inability of a bisexual flower to produce functional male gamete or viable zygote attributed to nuclear genomic origin (genomic male sterility) (g mst; Ariyanayagam *et al.*, 1993) or to nuclear and cytoplasm factors (cytoplasmic-genetic male sterily, g-c mst). Reddy *et al.* (1978) made the first serious attempt at ICRISAT to search a male sterile system that could be used in hybrid production technology. In pigeonpea, two types of g mst, namely, ms1 (Reddy *et al.*, 1978) and ms2 (Saxena *et al.*, 1983) were found and are governed by single

recessive genes. The inheritance of g-c mst is non-Mendelian, as the transfer of cytoplasm is through the maternal parent. In pigeonpea, natural outcrossing was noticed as early as 1919 (Howard *et al.*, 1919) but its utilization in commercial hybrid breeding program was ruled out (Singh *et al.*, 1974) mainly due to the nonavailability of male sterility. With the identification of g mst and g-c mst commercial utilization of the heterosis in pigeonpea was possible. In pigeonpea, male sterility can be induced through crosses, through chemicals such as streptomycin sulfate (SS), sodium azide (SA), Terramycin, ethylmethane sulfonate (EMS), through γ-rays, and through wide hybridization.

Hybrids produced using genetic male sterile lines were not successful on a commercial scale because of high labor costs and skill requirements among seed producers. This drawback was over come by CMS-based hybrid production introduced by ICRISAT. The CMS technology involves crossing the wild relative of pigeonpea with the cultivated variety and producing cytoplasmic male sterile plant through backcrosses. The male sterile progeny resulting from this cross is then crossed with other fertile restorer lines, resulting in all fertile offspring. CMS-based hybrids produce 30% more root mass than other varieties, which was a significant achievement in case of pigeonpea as it is a semi-arid crop (Saxena *et al.*, 2005).

Experimental hybrids developed by using CMS lines have demonstrated a yield advantage of over 25% (Saxena, 2004). It is a known fact that for a long-term commercially viable hybrid breeding program both genetic as well as cytoplasmic diversity are essential. For utilizing this new CMS source in a practical hybrid pigeonpea breeding program, the male sterility maintainers need to be identified among the cultivated types and this can be achieved by crossing a number of genetically diverse pigeonpea lines with the male sterile genotypes that should be followed by backcrossing and selection.

The magnitude of heterosis in pigeonpea is more or less similar to those of other crops such as maize, cotton, rice, millet, and sorghum (Saxena, 2004). Heterosis in pigeonpea could be exploited commercially if a grower-friendly mass-scale hybrid seed production technology is developed. So far, four wild relatives of pigeonpea have been successfully used to breed CMS systems for developing commercial hybrid pigeonpea breeding technology. These are: *C. sericeus* (Benth. ex Bak.) van der Maesen comb. nov. (Saxena *et al.*, 2002), *C. scarabaeoides* (L.) Thou. (Tikka *et al.*, 1997; Saxena and Kumar, 2003), *Cajanus volublis* Blanco (Wanjari *et al.*, 2001), and *C. cajanifolius* (Haines) Van der Maesen comb. nov. (Saxena, 2004). Very recently, Saxena *et al.* (2005) reported a new source of CMS developed by using the cultivated pigeonpea as the female parent and one of its wild relative *C. acutifolius* as the pollen donor and it is the first report in pigeonpea where CMS has been developed using the cytoplasm of cultivated pigeonpea.

So far four pigeonpea hybrids based on genetic male sterility were released in India and they are as follows:

ICPH 8: The world's first pigeonpea hybrid ICPH 8 (MS Prabhat DT × ICPL 169) was released by ICRISAT and ICAR in 1991. ICPH 8 was superior to controls UPAS 120 and Manak by 30.5% and 34.2%, respectively.

PPH 4: It was released in 1993 by Punjab Agricultural University (PAU), Ludhiana (Verma and Sindhu, 1995). PPH 4 (MS Prabhat DT × AL 688) recorded 32.1% higher yield than the best national check, UPAS 120. These early maturing pigeonpea hybrids with high yield potential are highly suitable for pigeonpea-wheat cropping system in the irrigated areas of northern India.

COPH 1 and COPH 2: In 1994, a short-duration hybrid IPH 732 (MST-21 × ICPL 87109) was released by Tamil Nadu Agricultural University (TNAU), Coimabtore as COPH 1 that recorded 32% higher yield over control VBN 1 (Murugarajendran *et al.*, 1995). In 1997, TNAU released another pigeonpea hybrid COPH2 (Ms CO 5 × ICPL 83027), which outyielded COPH 1 and CO 5 by 13% and 35%, respectively.

AKPH 4104 and AKPH 2022: They were released by Punjabrao Krishi Vidhyapeeth, Akola. AKPH 4104, released for central zone, is a short-duration hybrid gave 64% higher yield than control UPAS 120. AKPH 2022 is a medium-duration hybrid released for Maharastra state that recorded 34.9%, 28.2%, and 25.2% more yields than controls BDN 2, C 11, and ICPL 87119, respectively.

At ICRISAT, three CMS lines are being maintained. They are CMS 85010, a short-duration line with determinate growth habit; CMS 88034 is a nondeterminate short-duration type while CMS 13092 has genome from African germplasm and it belongs to long-duration group.

1.5.3 Embryo rescue

Wild relatives of crop plants comprise an important germplasm resource for plant improvement (Davey *et al.*, 1994). Crosses between distantly related plants are generally unfruitful because of the abortion of embryos on the mother plant. These embryos can be precociously excised and cultured *in vitro* (Monnier, 1990). By using this technique, a large number of hybrid plants have been obtained and several genetic characteristics have been transferred in grain legumes, such as hybrids produced in *Arachis* (Bajaj, 1990) and *Glycine* (Grant, 1990).

Mallikarjuna *et al.* (2006) reported successful generation of backcross progeny by the use of *in vitro* techniques and conventional backcross program. This is the first report in pigeonpea where an incompatible wild species from tertiary gene pool, such as *C. platycarpus*, has been successfully crossed with cultivated pigeonpea and fertile hybrids and backcross progeny obtained. When using wild species from the tertiary gene pool, it is usually necessary to use embryo rescue techniques at least once to obtain hybrid plants. But in pigeonpea using embryo rescue method twice, and increasing the ploidy ($2n = 44$) hybrids were produced between the incompatible cross of *C. platycrapus* with cultivated *C. cajan*. The study shows that it is possible to transfer important traits such as resistance to phytophthora blight from *C. platycarpus*, although it is distantly related to cultivated pigeonpea.

2. TRANSGENIC PIGEONPEA

The genetic transformation of pigeonpea plants involves the stable introduction of functional genes into the nuclear genome of cells capable of giving rise to a whole plant. Despite significant advances over the past decade, the development of efficient transformation methods can take many years of painstaking research. Transformation efficiencies, frequently, are directly related to the tissue culture responses and therefore, highly regenerative cultures are often transformation competent. Direct regeneration is preferred to indirect regeneration as the length of callus phase is negatively correlated with regeneration ability, where the somaclonal variation can also influence phenotype of the regenerated shoots (Fontanna *et al.*, 1993). In pigeonpea only few reports are available on genetic transformation and they are reviewed here.

2.1 Donor Genes

Gene cloning is the process of isolation and multiplication of an individual gene sequence by insertion of that sequence into a bacterium where, it can be replicated. Vectors used in cloning have been specially developed by adding certain features like: reduction in size of vector to a minimum; introduction of selectable markers and synthetic cloning or polycloning sites; incorporation of axillary sequences, etc. The genetic transformation generally involves two genes, namely, transgene that should be integrated in plant genome and expressed in the transgenic plant and the other is selectable marker gene. Each of the two transgenes should thus have their own promoter and termination sequences.

For producing pigeonpea transgenics for insect resistance, Lawrence and Koundal (2001) used the binary vector construct (pCPI) cloned in Bin19 having the cowpea protease inhibitor gene. Similarly, transgenic pigeonpea plants for oral vaccines were produced by using the recombinant binary vector pBI H carrying the *hemagglutinin* gene (*H*) for rinderpest virus and pseste des petitis ruminanats virus (PPRV) under the control of a cauliflower mosaic virus (CaMV 35S) promoter and *nos* polyadenylation sequence, and *npt*II as a marker gene under the control of the *nos* promoter and polyadenylation sequences (Satyavathi *et al.*, 2003; Prasad *et al.*, 2004). Here, the *H* gene was cloned initially in pBI 121 by replacing the *uidA* gene and mobilized to EHA 105 *Agrobacterium* strain. Pigeonpea transformation for disease resistance has been carried out with a disarmed C-58 strain of *Agrobacterium tumefaciens* harboring a binary plasmid pCAMBIA 1302: *RChit* (Kumar

et al., 2004b). Here, a rice chitinase (*Rchit*) gene driven by CaMV 35S promoter and a CaMV 35S poly-A sequence was subcloned into pCAMBIA 1302 vector to produce a pCAMBIA 1302:*RChit* binary plasmid. It also contained the hygromycin phosphotransferase (*hpt*) gene (used as a selectable marker) and the green fluorescent protein (*gfp*) gene (used as reporter gene) under the control of CaMV 35S promoter and CaMV 35S poly-A sequences.

Surekha *et al.* (2005) used the hybrid endotoxin Cry1E-C by replacing 530–587 amino acid residues of Cry1Ea protein with 70 amino acid region of Cry1Ca in domain III for pigeonpea transformation. The binary vector pPK 202 carried the *cry1E-C* and *npt*II genes driven by CaMV 35S promoter and *nos* polyadenylation sequence. Very recently, Sharma *et al.* (2006a) also reported pigeonpea transformation using *Bt cry1Ab* gene cloned into the binary vector pHS723 by blunt end ligation, where CaMV 35S promoter and polyadenylation sequence had driven the *cry1Ab* gene. The plasmid also contained fused *uid*A and *npt*II genes as reporter and marker genes, respectively. These genes were driven by double enhanced CaMV 35S promoter and poly-A sequence.

2.2 Methods of Genetic Transformation

Transformation of plants involves the stable introduction of DNA sequences usually into the nuclear genome of cells capable of giving rise to a whole transformed plant. Transformation without regeneration and regeneration without transformation are of limited value. Transformation in pigeonpea has been feasible using both *Agrobacterium* and biolistics mode of transformations but transgenic plant targeting a trait has been produced only using *Agrobacterium* mode of transformation. *A. tumefaciens* is a soil-borne bacterium that has been implicated in gall formation at the wounded sites of many dicotyledonous plants. To date, numerous strains of *A. tumefaciens* have been isolated and characterized, but only a few of these have been modified for use in the transformation of higher plants (Muthukumar *et al.*, 1996; Jaiwal *et al.*, 1998; Yan *et al.*, 2000; Krishnamurthy *et al.*, 2000).

The recent developments in genetic transformation in pigeonpea have emboldened researchers to pursue the development of transgenic pigeonpea plants resistant to various diseases, and insect pests (Satyavathi *et al.*, 2003; Kumar *et al.*, 2004b; Surekha *et al.*, 2005; Sharma *et al.*, 2006a). In most of the available reports in transformation of pigeonpea (Arundhati, 1999; Geetha *et al.*, 1999; Satyavathi *et al.*, 2003; Kumar *et al.*, 2004a, b), co-cultivation for 48 h in nonselective shoot regeneration medium proved good. However, Sharma *et al.* (2006a) reported that co-cultivation of the axillary meristem explants for 72 h was useful in obtaining a high frequency of stable transformants. The *Agrobacterium* strains that have been successfully used for pigeonpea transformation are based on chromosomal backgrounds of strains LBA4404 and C58 with a wide range of Ti and binary plasmids. Strains such as LBA4404, C58, EHA105, GV3101, and GV2260 and binary vectors like pBI121, pCAMBIA1301, pBAL2, and pHS723 were used in *Agrobacterium*-mediated transformation of pigeonpea (Arundhati, 1999; Geetha *et al.*, 1999; Lawrence and Koundal, 2001; Satyavathi *et al.*, 2003; Prasad *et al.*, 2004; Kumar *et al.*, 2004a, b; Singh *et al.*, 2004; Sharma *et al.*, 2006a, b).

Factors like growth phase of the *Agrobacterium* culture, co-cultivation medium, time period of co-cultivation, temperature during co-cultivation, addition of *vir*-inducing chemicals in the medium, promoters, and explants used etc. effects the *Agrobacterium*-mediated genetic transformation in pigeonpea. Addition of chemicals that induce *vir* genes in co-cultivation media is common practice in genetic transformation of many crop species especially monocots, however, the liberation of phenolic compounds by the explant itself is enough to favor the *Agrobacterium* infection in pigeonpea (Sharma *et al.*, 2006a).

Although, pigeonpea was considered to be recalcitrant for long, recent reports on its genetic transformation with convincing molecular evidence indicates that the *Agrobacterium*-mediated genetic transformation is feasible in pigeonpea. Lawrence and Koundal (2001) have used *Agrobacterium* strain GV2260 carrying the *npt*II and cowpea protease inhibitor genes on a binary vector to infect the embryonal axes of pigeonpea and obtained multiple shoots following callus proliferation on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP) ($2\,mg\,l^{-1}$) and indole 3-acetic acid (IAA)

(0.2 mg l^{-1}) using this method a transformation frequency of less than 1% was obtained. Following this, Satyavathi *et al.* (2003) reported the successful recovery of pigeonpea transgenics for protective *H*-antigen gene (Haemagglutinin gene) through direct regeneration of cotyledonary node and embryonic axis after infecting with the virulent strain EHA105 of *A. tumefaciens* that harbored the binary plasmid pBI121 carrying the *H* antigen of rinderpest virus and *npt*II genes. The bacterial culture at late log phase with an O.D. 0.6 at 260 nm and a co-cultivation period of 48 h was used in this study. Similarly, Prasad *et al.* (2004) produced transgenics expressing the HN protein of PPRV through *Agrobacterium*-mediated genetic transformation of cotyledonary node explants of pigeonpea. Kumar *et al.* (2004a) reported stable transformation of pigeonpea by using cotyledoanry nodes from *in vitro*-grown seedlings of pigeonpea. Co-cultivation of the explants with strain C58 of *A. tumefaciens* harboring the binary plasmid pCAMBIA1301 carrying *uidA* and *npt*II as reporter and selectable marker genes, respectively and rice chitinase gene as the candidate gene at late log pahse with an O.D. of 0.6 and co-cultivtaion for 48h resulted in stable transformation with 45% transformation efficiency. Pigeonpea transgenics resistant to *Spodoptera litura* were developed by Surekha *et al.* (2005) through direct regeneration of embryonal axis after infecting with the virulent strain GV2260 harboring a modified binary vector pPK202 carrying a synthetic *cryIE-C* gene under a constitutive 35S promoter and the marker gene neomycin phosphotransferase II (*npt*II). A co-cultivation period of 72 h was reported for transformation with *Agrobacterium* and obtained a transformation efficiency of 15%. More recently, Sharma *et al.* (2006a) reported the recovery of pigeonpea transgenics for insect resistance through direct organogenesis of axillary bud following 72 h co-cultivation with *A. tumefaciens* strain C58 harboring the binary plasmid pHS 723 having codon-optimized *cry1Ab* and fused *npt*II and *uid*A genes.

2.3 Selection of the Transformed Tissues

A successful gene transfer does not guarantee stable integration and expression of the foreign gene, even by using signals for the regulation of transgene expression. Antibiotic resistance genes allow the transformed cells expressing them to be selected from the populations of nontransformed cells. The population of cells that has been transformed and expresses a resistance gene is able to neutralize the toxic effect of the selective agent, either by detoxification of the antibiotic through enzymatic modification (Evans *et al.*, 1996; Joersbo *et al.*, 1998; Wang *et al.*, 2000; Jaiwal *et al.*, 2002) or by evasion of the antibiotic through alteration of the target (Jaiwal *et al.*, 2002). Effectiveness of a particular antibiotic resistance system depends mainly on elements such as, selective agent, explant used, and selectable marker genes. Amongst the most widely used antibiotic resistance genes as selectable markers, neomycin phosphotransferase II (*npt*II; Bevan *et al.*, 1983; Herrera-Estrella *et al.*, 1983) and hygromycin phosphotransferase (*hpt*; Van den Elzen *et al.*, 1985; Waldron *et al.*, 1985) are most common. Other selectable marker genes like gentamycin acetyltransferase (*accC3*) resistance, bleomycin and phleomycin resistance have also been employed, but are not used for routine transformations (Roa-Rodriguez and Nottenburg, 2003).

In pigeonpea so far only *npt*II and *hpt* genes have been employed for selecting the transgenic plants from untransformed ones. Concentration of the kanamycin used for selection of the transformants varied based on the explant used and mode of regeneration. For effective selection of transformants 50 mg l^{-1} kanamycin in the medium was reported by Lawrence and Koundal (2001) and Satyavathi *et al.* (2003). Whereas, 75 and 125 mg l^{-1} kanamycin concentrations were reported by Surekha *et al.* (2005) and Sharma *et al.* (2006a), respectively. Similarly, 5 mg l^{-1} hygromycin as selection pressure for selecting the transgenics was reported by Kumar *et al.* (2004b).

2.4 Regeneration of Whole Plants

Recent advances in plant tissue culture techniques have been exploited *in vitro* regeneration of pigeonpea plants. Like any other crop, genetic engineering of pigeonpea requires reproducible tissue culture protocols. Though pigeonpea is a recalcitrant crop, ample reports on its regeneration through tissue culture are available (Kumar *et al.*,

1983, 1984; Shivaparakash *et al.*, 1994; Mohan and Krishnamurthy, 1998; Geetha *et al.*, 1999; Singh *et al.*, 2002; Dayal *et al.*, 2003; Thu *et al.*, 2003; Sharma *et al.*, 2006a). Regeneration in pigeonpea is by development of shoot buds by organogenesis from areas surrounding a meristem such as cotyledonary nodal meristem (Mehta and Mohan Ram, 1980; George and Eapen, 1994; Geetha *et al.*, 1998) and rarely through somatic embryogenesis (Patel *et al.*, 1994). However, only a few protocols could be successfully utilized in genetic transformation studies (Lawrence and Koundal, 2001; Satyavathi *et al.*, 2003; Kumar *et al.*, 2004b; Surekha *et al.*, 2005; Sharma *et al.*, 2006a, b).

Direct regeneration systems have advantages, due to the rapidity of morphogenesis and no requirement of frequent subculture. Besides, *de novo* production of shoot primordial is extremely rapid and initially synchronous with the period of cellular differentiation. Such a regeneration system favors easy accessibility for *Agrobacterium*-mediated genetic transformation in pigeonpea. Lawrence and Koundal (2001) reported indirect regeneration from callus obtained from the embryonal axes when cultured on MS medium fortified with 2 mg l^{-1} BAP. This callus was used for infecting with *Agrobacterium* strain GV2260 containing the *CPTI* gene. For shoot initiation and development, the infected calluses were transferred to MS basal medium with 2 mg l^{-1} BAP, 0.2 mg l^{-1} IAA, and 50 mg l^{-1} kanamycin. Rooting of the regenerated shoots was accomplished on half-strength MS medium containing 2% sucrose, 0.7% agar, and 0.5 mg l^{-1} IBA (indole 3-butyric acid). However, this system resulted in a transformation efficiency of less than 1%.

Direct organogenesis from cotyledonary node and embryonic axes was obtained by culturing on MS media fortified with 8.87 μM BAP for initiation of shoot buds and 2.22 μM BAP for shoot elongation (Satyavathi *et al.*, 2003). And for rooting MS media supplemented with 1.48 μM IBA was used. Selection was applied after two days of co-cultivation with the *Agrobacterium* culture containing the *H*-gene. Using this method they obtained transformation frequency of 67% in cotyledonary node explants and 51% in embryonal axes, which was calculated as the number of explants survived the selection pressure. The same protocol was used by Prasad *et al.* (2004) in producing pigeonpea transgenics expressing heamagglutinin- neuraminidase gene.

Kumar *et al.* (2004a) reported direct regeneration from cotyledonary nodal explants on MS medium supplemented with 2 mg l^{-1} BAP. Further elongation of these shoots was obtained by culturing on MS medium supplemented with 0.5 mg l^{-1} GA_3 (gibberellic acid) and rooted on root induction medium consisting of MS medium with 1 mg l^{-1} IBA. Rooted plants were transferred to pots containing a 1:1 mixture of sand and soil and incubated for 1 week for acclimatization (by covering with a plastic bag initially and gradually exposing the plant to the open environment) prior to transfer to a glasshouse, thus obtaining a transformation frequency of 42.5%. Surekha *et al.* (2005) achieved regeneration from embryonal segments when cultured on MS medium fortified with 2 mg l^{-1} BAP. The same medium was used for further development of shoots from embryonal axes after co-cultivation with *Agrobacterium* containing the *cry1E-C* gene. Elongation of the developed shoots was obtained by culturing on MS medium fortified with 1 mg l^{-1} BAP, 3 mg l^{-1} GA_3, and 0.1 mg l^{-1} NAA (α-naphthaleneacetic acid). Elongated kanamycin (75 mg l^{-1}) resistant shoots were subsequently rooted on MS medium supplemented with 1.0 mg l^{-1} NAA and later transferred to sterile vermiculite for hardening followed by transfer to the transgenic green house.

Recently, a highly efficient and genotype-independent direct organogenesis from the meristematic tissue developed from the axillary bud region of *in vitro*-grown seedlings of pigeonpea has been reported by Sharma *et al.* (2006a) by culturing the meristematic tissue on MS medium supplemented with 22.0 μM BAP for induction and development of shoot buds. Selection and enrichment of the transformed cells was initiated by applying a selection pressure of 125 mg l^{-1} kanamycin in the media after 2 weeks of infection with *Agrobacterium*. For elongation developed shoots were transferred to Shoot elongation medium containing MS with 0.5 μM GA_3. Rooting of the elongated shoots were obtained by giving pulse treatment with 25 μM IAA and culturing on plane MS medium with 1% sucrose (Figure 1a–k). Using this method 60% transformation efficiency was obtained that was calculated based on the positive gene integration.

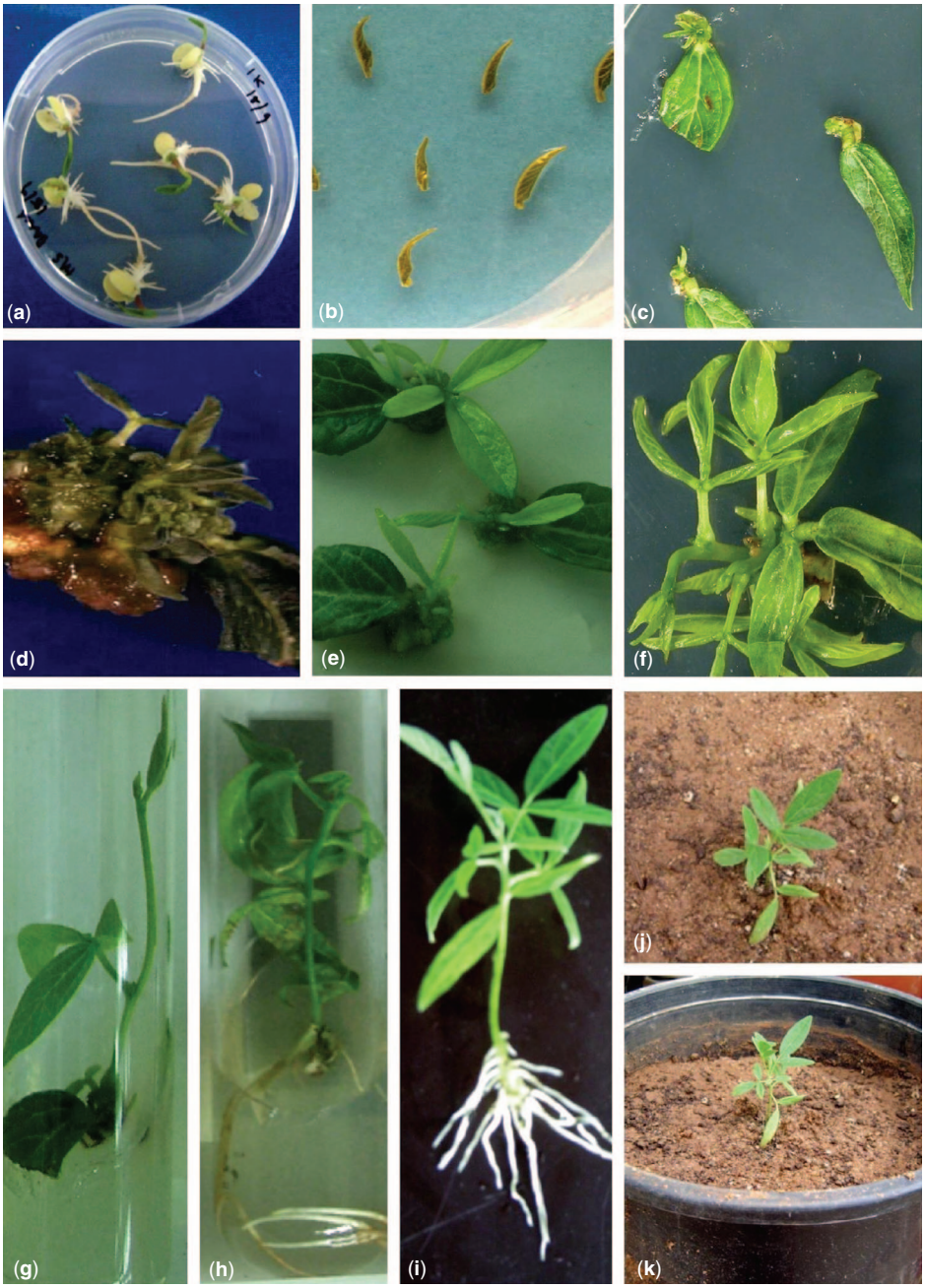

Figure 1 Regeneration of multiple shoots from leaf explants derived from *in vitro* germinated seedlings of pigeonpea, *Cajanus cajan* L. (a) Seeds showing germination on MS medium after 7 days of culture, (b) five-day-old leaf explants on MS medium supplemented with 5.0 μM BAP and 5.0 μM kinetin (shoot induction medium (SIM)) after carefully cutting and removing the preformed meristamatic region, (c) swelling of the petiolar region after 5 days resulting in the induction of adventitious shoot buds, (d, e) leaf explant with half-cut lamina showing multiple shoot initiation and development on reduced SIM (MS+ 2.5 μM BA+ 2.5 μM kinetin), (f) multiple shoot formation after 21 days of culture from the petiolar cut end, (g) explant with elongated shoot in shoot elongation medium (SEM) containing MS supplemented with 0.58 μM GA_3, (h) elongated shoot after pulse treatment with IAA (11.2 μM) showing rooting on root induction medium (RIM), (i) well-developed plantlet just before its transfer to small pots, and (j, k) fully established healthy seedlings transferred into bigger pots (13 in.) containing sand:soil mixture.

2.5 Testing of Transgenic Plants

Selection and growth of plant cells on selective media provide initial phenotypic evidence for transformation. After selection, the putative transgenic shoot is propagated *in vitro* followed by rooting and transfer to the containment glasshouse for further evaluation and production of seeds from subsequent sexual generations. Molecular evidences are essential to further confirm the integration and expression of transferred genes followed by genetic characterization (Birch, 1997).

Transgenes are expected to behave as dominant genes due to their hemizygous state in receipient genome and thus segregate as dominant loci in a typical 3:1 Mendelian ratio (Campbell *et al.*, 2000). Successful genetic transformation of any plant involves not only the production of primary transformants showing stable expression of inserted gene but also the inheritance of introduced trait. Skewed segregation of the introduced genes, during meiosis leading to non-Mendelian inheritance may be caused due to various reasons, such as linkage to a recessive lethal gene, mutational effect of transfer DNA (T-DNA) insertion, and chromosomal rearrangement.

Satyavathi *et al.* (2003) were successful in producing oral vaccines in pigeonpea for rinderpest virus (RPV) disease in cattle by using surface glycoprotein *H*-gene of RPV. Presence and the expression of the candidate and marker genes were confirmed by polymerase chain reaction (PCR) analysis and reverse transcripase-PCR (RT-PCR) analysis, respectively. Southern hybridization proved the integration of the genes with one to more than three copies of the gene. Expression at the protein level was confirmed by Western analysis and enzyme-linked immunosorbent assay (ELISA) for H-protein and was reported as 0.12–0.49% of the total soluble leaf protein in pigeonpea. Transgenic plants were analyzed till T_1 generation and observed 3:1 ratio of segregation of the genes. Oral vaccines were also produced in pigeonpea for another cattle disease, Peste des petits ruminants (PPR) by Prasad *et al.* (2004) using *HN* gene. Presence and integration of the transgene was confirmed by PCR and southern analysis and the protein expression through Western blot analysis.

Kumar *et al.* (2004b) produced pigeonpea transgenics with rice chitinase gene. The plants were characterized by PCR and RT-PCR analysis for the confirmation of presence and expression of the gene. Plant DNA isolated was also subjected to southern hybridization for copy number and it varied from one to four. Plants were advanced to T_1 generation and the inheritance pattern followed Mendelian segregation.

Surekha *et al.* (2005) reported the molecular characterization of the produced pigeonpea transgenics using PCR and Southern blot analysis for *cry1E-C* gene. Protein analysis was carried out by Western blot analysis and the inheritance pattern followed Mendelian inheritance in T_1 and T_2 generations. Bioassays were performed using first and second instars of *S. litura* in T_1 and T_2 generations, which showed a varied response (60–80% mortality) for resistance to *Spodoptera*. Besides, Sharma *et al.* (2006a) produced pigeonpea transgenic plants resistant to *H. armigera* using *cry1Ab* gene. PCR, Southern, and RT-PCR analysis were used for confirming the presence, integration, and expression at RNA level. Southern hybridization data had shown one to two copies of the integrated gene in the transgenic plants. All plant parts were used for ELISA analysis to check the expression of the *cry1Ab* gene. The transgene protein level reported was as high as 0.1% in flowers and as low as 0.025% in leaves. The segregation of the genes were tested till T_3 generation and showed Mendelian inheritance (3:1). In all the available reports of pigeonpea transgenics (Table 2) there are no indication of adverse effects of genetic transformation methods on the growth, yield, and quality of the transgenic plants.

2.6 Regulatory Measures

In pigeonpea, no reports are so far available on commercially grown transgenics. At ICRISAT, transgenic pigeonpea plants carrying either the *cry1Ab* (unpublished data) or the *cry1Ac* (Sreelatha, 2006) genes were evaluated under contained field conditions with the approval of the Institutional Biosafety Committee (IBSC) of ICRISAT and the Department of Biotechnology, Government of India. However, these transgenic events are still under evaluation and validation.

Table 2 List of transgenic plants produced in pigeonpea

Explant	Mode of regeneration	Genes used	Mode of transformation	*Agrobacterium* strain used	References
Leaf disks	Direct organogenesis	*npt*II and *uid*A	*Agrobacterium*	LBA 4404	Arundhati, 1999
Shoot apices and cotyledonary node	Direct organogenesis	*npt*II and *uid*A	*Agrobacterium*	LBA 4404	Geetha *et al.*, 1999
Embryonic axes	Callus	*CPTI*, *npt*II	*Agrobacterium*	GV 2660	Lawrence and Koundal, 2001
Embryonic axes and cotyledonary node	Direct organogenesis	*H*-gene and *npt*II	*Agrobacterium*	EHA 105	Satyavathi *et al.*, 2003
Leaf	Direct organogenesis	*npt*II and *uid*A	Biolistics	–	Dayal *et al.*, 2003
Cotyledonary node	Direct organogenesis	*npt*II and *uid*A	Biolistics	–	Thu *et al.*, 2003
		H-N gene and *npt*II	*Agrobacterium*	GV3 101	Prasad *et al.*, 2004
		hpt and *uid*A	*Agrobacterium*	C58	Kumar *et al.*, 2004a
		R chit, *npt*II and *uid*A	*Agrobacterium*	C58	Kumar *et al.*, 2004b
Shot apices	Direct organogenesis	*hpt* and *uid*A	*Agrobacterium*	C58	Singh *et al.*, 2004
Embryo axis	Direct organogenesis	*cry1E-C* and *npt*II	*Agrobacterium*	GV 2260	Surekha *et al.*, 2005
Axillary meristem	Direct organogenesis	*cry1Ab* and *npt*II	*Agrobacterium*	C58	Sharma *et al.*, 2006a

3. FUTURE ROAD MAP

3.1 Expected Products

The majority of protein food in India comes from pulses, grown invariably under unfavorable growing conditions that result in low productivity. Pigeonpea suffers from damage caused by biotic and abiotic stresses and due to these constraints it had a low compound growth rate of 0.8% in production between 1950 and 2004 (Ahlawat *et al.*, 2005). To combat the biotic stresses farmers rely on application of insecticides. Unfortunately, chemical control of insect pests is under increasing pressure due to environmental degradation, adverse effects on human health and other organisms, eradication of beneficial insects, and development of pesticide resistant insects. Improvement of pigeonpea through conventional breeding methods was slow though its wild varieties were rich source for insect resistance. Together with improved techniques for plant genetic analysis and engineering, concepts of exploiting transgenic plants have gained increasing scientific and economic importance. Even transgenic technology application in improving this crop has started in recent past as it was said to be a recalcitrant crop. Quality of pigeonpea in terms of nutrition, resistance to biotic and abiotic stresses has to be further probed ahead. Modern biotechnological tools in combination with traditional technologies hold great promise for augmenting agricultural productivity in quantity as well as quality. Here under are some of the important traits that can be improved in pigeonpea as well as extended in producing functional recombinant proteins using transgenic technology.

3.1.1 Insect resistance

Insect pest menace is the major factor that destabilizes crop productivity in agricultural ecosystems. A survey conducted among plant breeders, pathologists, and entomologists shows that breeding for resistance to insect pests is at the top of their priority list for many important crops

(Ranjekar *et al.*, 2003). Insect pest management by chemicals has brought about a considerable protection to crop yields over the past five decades. The pod borer, *H. armigera* is commonly regarded as the key pest throughout Africa and Asia on pigeonpea. It is particularly damaging on early formed pods. In many parts of India the podfly, *M. obtuse*, takes over as the dominant pest later in the season. In pigeonpea, the losses due to *Helicoverpa* have been estimated at US$ 317 million in the SAT, and possibly over US$ 2 billion on different crops worldwide annually (Sharma *et al.*, 2001). Losses due to pod fly damage have been estimated to be US$ 256 countries in Asia, Africa, and the Caribbean for food, million annually (ICRISAT, 1992). To overcome these losses, farmers resort to excessive use of pesticides. Crop surveys have indicated that before 1975, only 20% of the pigeonpea farmers were using insecticides, but by 1993, 100% of the farmers have adopted the use of chemicals to control *H. armigera* in India. In pigeonpea, one larva per plant reduces 4.95 green pods, 7.05 dry pods, 18.01 grains, 3.79 g pod weight and 2.05 g grain weight. Wild varieties are a precious source of resistance for insects attack in pigeonpea but genetic improvement of pigeonpea has been restricted due to the nonavailability of better genetic resources and strong sexual barriers with the wild species.

Insect resistant plant varieties, using ∂-endotoxins of *Bacillus thuringiensis* (*Bt*), have been produced in several important crops such as tobacco, tomato, cotton, rice, brinjal, maize, broccoli, oilseed rape, soybean, walnut, poplar, sugarcane, apple, potato, groundnut, sweetpotato, chickpea, alfalfa, etc. (Hilder and Boulter, 1999; James, 2002; Sharma *et al.*, 2004). Of the US$ 10 billion spent annually on insecticides worldwide, it has been estimated nearly US$ 2.7 billion could be substituted with *Bt*-based biotechnology applications (Krattiger, 1997). There is significant increase in global area under transgenic crops from 1.7 million hectares in 1996 to 90 million hectares in 2005, in which *Bt* crops share was 25% of the total area (James, 2005).

Since the first report on the introduction of *Bt*-derived *cry* genes into tobacco (Barton *et al.*, 1987) and tomato (Fischhoff *et al.*, 1987; Vaeck *et al.*, 1987), there has been a rapid increase in the transformation of other crop plants to achieve resistance against insect pests and were successful (Stewart *et al.*, 1996; Nayak *et al.*, 1997; Adamczyk *et al.*, 2001a, b; Sanyal *et al.*, 2005). At least 10 different genes encoding different *Bt* toxins, namely, *cry1Aa, cry1Ab, cry1Ac, cry1Ba, cry1Ca, cry1H, cry2Aa, cry3A, cry6A, and cry9C* have been engineered into different crop plants (Schuler *et al.*, 1998). All these transgenics showed resistance to the respective pests. These results show that *Bt* gene is an efficient insecticidal gene that can be deployed for producing transgenic pigeonpea plants for pest resistance.

In pigeonpea, though transgenic plants with *Bt* and *CPTI* genes are available to combat the insect pest *H. armigera* more events with high expression need to be produced. Apart from *Bt* and protease inhibitor genes, insecticidal chitinase also been shown to be important in controlling the devastating pest, *Helicoverpa*, by dissolution of the chitin, an insoluble structural polysaccharide that occurs in the exoskeleton and gut lining of insects. Gene pyramiding with two different insecticidal genes and tissue-specific expression to reduce the risk of developing insect resistance are other attractive options to combat this pest and for durable resistance.

3.1.2 Fungal resistance

Fungal diseases involving 45 pathogens are known in pigeonpea and the most important and widespread disease is wilt (*F. udum*), favored by soil temperatures of 17–20 °C. It affects the plants at all stages of its development and in India it causes a loss of 5–20% and in severe case upto 50%. Recombinant-DNA technology allows the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant (Keen, 1999) or by choosing plant genes that intensify or trigger the expressions of existing defense mechanisms (Bent and Yu, 1999; Rommens and Kishmore, 2000). Transgenic pigeonpea for wilt resistance using rice chitinase gene has developed and are being evaluated for resistance against wilt at ICRISAT. But still much work has to be further done to develop transgenics with high expression of the gene.

3.1.3 Virus resistance

Viral diseases affect worldwide productivity of the economically important crops. In pigeonpea

the sterility mosaic disease considered as "green plague of pigeonpea" caused by sterility mosaic virus (SMD) is being recognized as a serious economic threat as it can cause complete crop failure if occurs early in the season. SMD infection at an early stage (<45 days old plants) results in a 95–100% loss in yield (Kannaiyan *et al.*, 1984; Reddy *et al.*, 1990), while losses from late infection (>45 days old plants) depend on the level of infection (i.e., number of affected branches per plant) and range from 26% to 97% (Kannaiyan *et al.*, 1984). Often the most significant weapons against viral diseases are cultural controls (such as removing diseased plants) and plant varieties bred to be resistant (or tolerant) to the virus, but they may not always be practical or available. There are mainly two approaches for developing genetically engineered resistance depending on the source of the genes used. The genes can be either from the pathogenic virus itself or from any other source. Substantial yield increase that was observed in field trails in some transgenic crop plants has clearly established the reliability of coat protein-mediated resistance (CPMR) as the most favored strategy to engineer resistance against many viruses. Apart from CPMR transgenics with replicase protein-mediated resistance is commonly produced for viral resistance and could be an option to develop pigeonpea transgenics with resistance to SMD.

3.1.4 Abiotic stresses

Drought, cold, heat, and salinity are the abiotic stresses that affect the pigeonpea yield. Besides, waterlogging, heavy rains and frost are very harmful for the crop. Hence, improvement of pigeonpea for tolerance to these abiotic stresses is very important as it increases the harvest index and ultimately the yield.

Though pigeonpea is classified as moderately sensitive to salinity (Keating and Fisher, 1985), development of salt-tolerant variety could be useful for Indian farmers as it is grown predominantly in the states of Uttar Pradesh, Gujarat, and Maharashtra, where more than 51% of the saline soils in India are located (Agarwal *et al.*, 1976). Moreover these states contribute about 85% of the total growing area and production of pigeonpea in India (Muller *et al.*, 1990). Also, pigeonpea is sensitive to photoperiod and temperature. Low temperatures affect the short duration variety whereas high temperature and photoperiod affects the yield in medium and long-duration variety rendering them to terminal drought. In cool areas, maturity in long-duration pigeonpea is accelerated and severe competition occurs between intercropped maize whose maturity is delayed and pigeonpeas resulting in yield reduction of both crops.

Importance of transgenic technology in improving salinity tolerance has been already proved in *Arabidopsis* by overexpressing *SOS1* gene by limiting Na+ accumulation in plant cells (Shi *et al.*, 2003). The overexpression of a *AtNHX1* and *H+−PPiase* genes in *Arabidopsis* (Apse *et al.*, 1999; Gaxiola *et al.*, 2001) and *AtnHX1* gene in tomato and canola resulted in transgenic plants that were able to grow in high salt concentrations (Zhang and Blumwald, 2001; Zhang *et al.*, 2001). It is proven that membrane lipids hold the key for improvement of photosynthesis under low temperature and high temperature stress conditions (Grover *et al.*, 2000). Betaine has been shown to accumulate in response to low and high temperature stress in higher plants, where it might play a role in protecting membranes and/or protein complexes (Zhao *et al.*, 1992; Yang *et al.*, 1996). Introduction of choline dehydogenase (*CDH*) gene that encodes betaine in tobacco resulted in low temperature tolerance a part from salt tolerance. Similarly, catalase in rice (Tanida and Saruyama, 1995; Tanida, 1996), *codA* gene in *Arabidopsis*, cDNA (complementary DNA) of chloroplast enzyme glycerol-3-phosphate acyltransferase in tobacco, and *Arabidopsis* have proven to impart resistance to various abiotic stresses including chilling and high temperatures. However, no transgenics are reported so far in pigeonpea for these traits. These results prove the feasibility of producing transgenics in pigeonpea for various abiotic stresses, which need to be improved for getting high yield varieties.

3.1.5 Biofortification

The efficiency of productivity depends on total nutrient content in the seed that meets the need of the population with minimal waste. Greater attention needs to be given to the pigeonpea to improve amino acids profiles, in particular to improve the level of sulfur-containing amino acids and to eliminate antinutritional factors.

Dissection of biosynthetic pathway and introducing appropriate genes or engineering entire biochemical pathway is generally applied for increasing the nutritional value of the crop. With similar strategies it should be possible to achieve similar increases in pigeonpea plants. Besides enhancing the nutritional value, transgenics produced for micronutrient deficiency such as Vitamin E can also produce antioxidants for industrial applications.

Biotechnology and genetic modification techniques are being optimized for the production and development of healthy foods, and improvement in the levels and activity of biologically active components in food plants (phytochemicals). The production of increased levels of β-carotene (provitamin A) in plants is especially important, as its precursor, lycopene has been shown to have physiological chemopreventive effects with regard to various cancers (Yan and Kerr, 2002). Furthermore, lycopene, commonly found in various carotenoids containing plants, such as tomatoes and carrots, is an essential ingredient in maintaining eye health and vision. β-carotene, α-carotene, β-cryptoxanthin are carotenes that are converted into Vitamin A or retinol in the body. Pigeonpea occupies an important place in human nutrition as a source of dietary proteins in several countries. Work has been initiated at ICRISAT in developing pigeonpea transgenics by using the phytoene synthase gene (*psy1*) that converts geranylgeranyl pyrophosphate to phyotene. Increase in the phyotene content during the biosynthetic pathway of carotenoids in turn increases the β-caretone level, which is a precursor of Vitamin A. Success in producing transgenic pigeonpea plants with high-level expression of phyotene synthase will have much to contribute to the malnourished population. Besides, pigeonpea contains the lowest amount of limiting sulfur amino acids, methionine, and cysteine among all important food legumes, implicating the importance of these amino acids in its protein quality improvement program. The functional importance of dietary methionine and cysteine lies in the intestinal growth and function, beyond its role as a precursor for protein synthesis. Cysteine has a key role in cellular antioxidant function, which is a determinant of cell proliferation and survival. Promoting work in this direction could be helpful in increasing the protein quality of the pigeonpea. With this in view, production of pigeonpea transgenics with *SSA* (sunflower seed albumin) gene that encodes methionine has been initiated at ICRISAT. Apart from this, considerable attention needs to be given to improve the quality of pigeonpea protein by reducing the polyphenolic compounds that are present in abundance that subsequently affects the activity of digestive enzymes.

3.1.6 Hybrid seed production

For hybrid seed production availability of cytoplasmic male sterile lines and restorer lines are necessary. The development of these lines through conventional breeding is a slow process that minimally requires several years of effort. In pigeonpea, using wide hybridization it was possible to produce CMS line and by screening large accessions, restorer lines were identified and were used to produce hybrids, which performed well in terms of biomass production and abiotic stress tolerance. But hybrid seed production was not to the expected level due to the high cost, poor in-built insect resistance, and management. Development of transgenics for CMS is possible as is the case in tobacco (Mouras *et al.*, 1999) where male sterility was induced by transferring the *u-atp9* (ATPase) gene. Also the development of transgenics by the introduction of transgenes in to the isogenic lines or in to the chloroplast of CMS line already available could reduce the risk of transgenes effect on pollinators such as honeybee and also could restrict the gene flow. Transgenic maintainer lines can be further utilized in enhancement of hybrid seed production with desired characters and also for development of inbred varieties.

Chloroplast engineering has been proposed as a safer approach to the containment of transgenes as chloroplasts are not transmitted in the pollen of flowering plants (Daniell and Khan, 2003; Ruiz and Daniell, 2005).

3.2 Addressing Risks and Concerns

The ethical dilemmas associated with the introducing of transgenic crops in the farming systems have divided both public and private researchers worldwide (Ortiz, 1998). This was not unexpected because the adoption of a new technology has

been always subject to distinct vision and ethical perspectives (Crouch and Ortiz, 2004). In times of severe global decline of biodiversity, proactive precaution is necessary and careful consideration of the likely expected effects of transgenic plants on biodiversity of plants and insects is mandatory (Wolfenbarger and Phifer, 2000; Velkov, 2001, 2003a, b).

Transgenic crops represent a powerful and profitable extension of conventional breeding methods in pigeonpea. However, challenge is to use this technology wisely, as part of a long-term strategy to improve human health, preserve biodiversity, and promote more sustainable agricultural practices in resource-poor countries. Enhancing the productivity of pulse legumes, such as pigeonpea, that are frugal in nature and are also important for nutritional security will be a possible way of addressing the additional problems of water deficits and high input costs in arid and SAT. Traditional cropping systems in pigeonpea include intercropping or mixed cropping (Aiyer, 1949; Acland, 1971; Osiru and Kibira, 1981) and crop rotation schemes for maintaining the soil fertility and minimizing erosion. Pigeonpea that is considered to be frugal in their requirement of water and fertilizers if made resistant to pests and pathogens using transgenic technology, could very well replace the cereals (maize, sorghum, pearlmillet, and finger millet), fiber, and other legume crops allowing proper crop rotation and ensuring a diversification. Similarly, transgenic technology can contribute toward enhancing productivity and yield stability of pigeonpea with low-water requirement resulting in a dramatic reduction in the overall dependence on groundwater for irrigation.

However, a major concern of the scientists and the environmentalists is that the transgenes could escape to related species by pollen flow and could convert wild relatives into "super weeds." However, reports on pigeonpea indicate that "genetic pollution" by transgenes escaping into landraces, primitive cultivars, and nontransgenic varieties through pollen dispersal has been recently focused as a major issue in the risk assessment of transgenic plants. However, this risk can be very well addressed by introducing the transgenes into the chloroplast genomes of plants. These organelles are inherited maternally and therefore, pollen-based dispersal is not possible.

Breeding for resistance to pests and pathogens has been a major area of research in pigeonpea breeding. There is a concern that widespread growing of *Bt* transgenics may lead to insects developing resistance to *Bt* proteins, thereby increasing criticisms on the use of one of the most potent but more environmental-friendly pesticidal tool. Most of the pigeonpea transgenics produced so far using *cry1* genes that are insecticidal only to selected groups of insect species (e.g., lepidoptera) and are unlikely to have direct effects on species outside this group. Although the extensive testing on nontarget plant feeding insects and beneficial species that have accompanied the long term and wide scale use of *Bt* plants has not detected significant adverse effects (reviewed by O'Callaghan *et al.*, 2005). However, species representing pollinators (honeybees), natural enemies (including predators and parasitoids), and detritovores are among the insects that should be subjected to elaborate tests with transgenic pigeonpea containing insecticidal genes. However, there is an increasing concern that other transgenic proteins with ranges of activity wider than those of *Bt* may have a greater chance of affecting natural enemies. Transgenic expression of non-native proteins in plants may lead to the concerns on potential for new allergens in genetically modified (GM) crops substantiate the need for the complete risk assessment of transgenic crops before commercialization (Prescott *et al.*, 2005). Hence, an objective assessment of the associated hazards needs to be carried out during feeding trials for testing immunogenic responses of the transgenic foods on the animal model systems before any commercialization. Besides, concerns on the possibilities of breakdown of resistance to pests and pathogens is always there even if the resistance is based on the use of the secondary gene pool or through the use of the tertiary gene pool using conventional breeding methods. Although, this itself can be counteracted by imparting a more durable resistance in crop plants by diversifying the resistance-conferring genes, i.e., using more than one gene in a variety and eventually stacking genes, which confer resistance through diverse mechanisms.

The possible risks posed by cross hybridization with wild relatives have been extensively explored (reviewed in Stewart *et al.*, 2003). However, the Nuffield Council on Bioethics suggests that introgression of genetic material into related

species in centers of crop biodiversity is an insufficient justification to rule out the use of GM crops in the such areas and the developing world (Nuffiel Council on Bioethics (a discussion paper), 1999). Nonetheless, banning of transgenic crops does not appear as a scientifically sound option because of the potential benefits derived from their utilization by farmers, for example, resistant or tolerant crops to abiotic and biotic stresses obtained through genetic engineering (Sharma *et al.*, 2002).

The field evaluation and risk assessment have to be performed according to the biosafety guidelines of the host country under the immediate guidance and supervision of the Institute Biosafety Committee. Assessment procedures are being harmonized internationally by various organizations (Levin and Strauss, 1993). Hence, the release of transgenic materials should take place only after establishing the utility of the material through very transparent and well-documented evaluations. In addition to the measurable parameters such as the crop performance, yield, fitness, invasiveness, rate of hybridization, the expanded risk equation now also includes nonquantifiable terms such as consumer choice, long-term agricultural policy, ethics, and societal responsibility to future generations.

The key to the future of genetically engineered pigeonpeas is firstly the public awareness toward the competent assessed of any risk associated with the transgenics and also that the safety has been ensured. With the development of high-throughput technologies in sequencing, it is now possible to mine genes of high agronomic value from the near and distant relatives of crop plants and to introduce these gene(s) into recipient crop varieties through the techniques of genetic transformation. Understanding the mechanisms of resistance and the mining of alleles that will confer resistance to pests and pathogens will be the most rewarding area for development of resistant pigeonpea cultivars through transgenic approaches

3.3 Expected Technologies

3.3.1 Clean transgenics

The antibiotic resistance genes are often of importance to select for transformants from nontransformants in the process of producing transgenic plants. But emergence of bacteria that are resistant to multiple or all antibiotics (Levy, 1997; Amtsblatt der Europäischen Gemeinschaften, 1998), transfer of resistance traits into weeds (Dale, 1992; Gressel, 1992) through cross-pollination with the related species and widespread distribution of resistance markers in to food products leading to horizontal transfer of resistance genes to gut bacteria, has increased the concern of antibiotic usage in transgenic plants. As these markers are used only as a tool of selection and as it does not code for any desirable traits the presence of these in transgenic plants is treated as a burden disturbing the genetic constituency of the plant and its wild varieties. Therefore, gene products need to be assessed for safety and environmental impact (Bryant and Leather, 1992; Gressel, 1992). Moreover, it is difficult to introduce a second gene of interest into a transgenic plant that already contains a resistance gene as a selectable marker because of limited availability of marker genes. The presence of multiple homologous sequences in plants enhances the likelihood for homology-dependent gene silencing (Matzke and Matzke, 1991), which could severely limit the reliable long-term use of transgenic crops. Therefore, it is necessary for scientists to look for alternatives for safer marker genes or elimination of the marker genes from transgenic plants to produce environmentally safe transgenic plants and pyramid a number of transgenes by repeated transformation (Yoder and Goldsbrough, 1994).

3.3.2 Marker-free transgenic plants

Marker-free transgenic plants can be obtained by using the site–specific recomabinase P1 Cre/lox (Yoder and Goldsbrough, 1994; Vergunst and Hooykaas, 1998; Vergunst *et al.*, 1998; Gleave *et al.*, 1999) system. Marker gene to be introduced into the plant cell if placed between two lox sites will be excised from the plant genome by the expression of *Cre recombinase*. This technology was successful in producing marker-free transgenics in tobacco (Gleave *et al.*, 1999; Jia *et al.*, 2006). Besides cre/loxP, *Zygosaccharomyces rouxii* R/*rs* (Onouchi *et al.*, 1991; Sugita *et al.*, 1999, 2000) and *Saccharomyces cerevisiae* Flp/*frt* (Kilby *et al.*, 1995; Lyznik *et al.*, 1996; Davies

et al., 1999; Luo *et al.*, 2000; Gidoni *et al.*, 2001) recombination systems are also in use for producing the marker-free transgenics. Marker-free transgenic plants can also be obtained by using *ipt* gene attached to *Ac* transposable element as a marker. The transgenic plants acquires abnormal phenotype called extreme shooty phenotype and looses its ability to root and therefore can be differentiated visually from nontransgenic plants. As the marker is attached to an *Ac* transposable element during the transposition process the *ipt* gene may transpose or become lost along with the *Ac* element and thus a normal, marker-free transgenic plant can be obtained. Co-transformation of desired gene and the marker gene on separate plasmids within the same *Agrobacterium* strain and selecting the transformants with both the genes unlinked is another option for obtaining marker-free transgenics. The two T-DNA binary vector systems (Komari *et al.*, 1996; Xing *et al.*, 2000; Matthews *et al.*, 1997; McCormac *et al.*, 2001; Miller *et al.*, 2002) represent a useful approach to generate selectable marker-free transgenics by co-transformation of the vector harboring two T-DNAs each bearing a marker gene. This system represents a valuable approach to generate selectable marker-free plants, with a consistent frequency seen among three elite cultivars of rice decreasing the plasmid backbone transfer, lowering the number of T-DNA copy integrations, and avoiding artifacts due to gene silencing (Sharma *et al.*, 2005).

3.3.3 Alternatives to antibiotic resistance markers

It is not possible to remove marker genes once they are integrated into a plant genome unless a particular mechanism for removal is incorporated along with the marker gene and the gene of interest at the time of the transformation. The removal prior to commercialization of marker genes, which are driven by plant promoters and are used for selection of plant cells, has become the aim of both consumers and industry. Markers that confer resistance to chemicals other than antibiotics, such as herbicides, and lethal concentrations of the amino acids lysine and threonine and/or markers that enable the plant cells to grow in the presence of unusual nutrients, including cytokinin, glucuronides, xylose or mannose, which will not allow nontransformed plant cells to grow can be the alternative selectable markers for plants. But expression of high levels of lysine and threonine causes abnormal growth in plant cells by interfering with amino acid biosynthesis and the presence of herbicide-tolerance markers may be undesirable. The relevant genes are therefore not suitable as marker systems.

Using a scorable marker gene could be an alternate for avoiding the usage of selcetable antibiotic marker genes. The reporter genes or scorable markers produce a visible effect, directly or indirectly, due to their activity in the transformed cells. Scorable markers like *uidA* (*gus*) gene, and *cat* gene are commonly used in transformaqtion experiments. However, destructive nature of β-glucuronidase (GUS) assay (Patnaik and Khurana, 2001) and presence of inhibitors of chloramphenicol-acetyltransferase (CAT) activity and endogenous CAT activity hampered the use of these as reporter genes (Patnaik and Khurana, 2001). Thus, to study the fate of introduced transgenes in living cells, vital reporter genes encoding for anthocyanin biosynthesis, green fluorescent protein, and firefly luciferase have been used successfully (Harvey *et al.*, 1999; Jordan, 2000). The delivery of a gene encoding mannose-6-phosphate isomerase and/or xylose isomerase allowing mannose and/or xylose to be metabolized in plant cells and the subsequent cultivation of those cells in a medium containing mannose and/or xylose as the sole source of sugar would allow only those cells that have taken up the gene to grow. Using mannose isomerase successful transformation of sugar beet (Joersbo *et al.*, 1998, 1999, 2000) and maize (Negrotto *et al.*, 2000; Wang *et al.*, 2000) was obtained. Using xylose isomerase, transgenics in potato, tomato, and tobacco were obtained with considerable transformation frequency (Haldrup *et al.*, 1998a, b). Another selectable marker gene that can be used is cyanamide hydratase (*Cah*) gene (Weeks, 2000). The *Cah* gene gives the transformed tissues the ability to grow on cyanamide-containing media by converting cyanamide into urea (which can be used as a fertilizer source).

Most alternatives are still in their development phase, are not widely available and will be difficult to implement in a less developed country. Alternative markers and marker removal systems

are being investigated in response to public concerns and to expand the number of tools available in plant molecular biology. Since the time for development of new alternative methods varies between different crops, it will be necessary to allow for a gradual transition to such technologies. It will also be critical to conduct safety assessments on new systems before they are used in products that are to be commercialized. Replacement of the technology, which makes use of antibiotic resistant marker gene such as *npt*II, will be desirable when the new technologies have ensured at least the same degree of scientific knowledge and confidence regarding their use as *npt*II gene and products containing it.

3.3.4 Choice of promoters

An efficient transformation system, in conjugation with the availability of a range of promoters with varied strengths and tissue specificities is critical to the success of transgenic approaches for crop improvement. An important aspect of transgenic technology is the regulated expression of the transgenes. Variation in transgene expression levels between different species and promoters may be due to different abundance of transcription factors, recognition of promoter sequences or intron splicing sites (Wilmink, 1995), or other factors. Therefore, increasingly, knowledge gained from genomics and postgenomics projects might provide information on designing of new targets for pigeonpea transformation. Establishment of transgenic systems for crops like pigeonpea requires genes of agronomic importance like those for insect resistance, abiotic stress tolerance, nutritional improvement, and male sterility. However, achieving a high expression of the introduced foreign gene in plant cells is still a challenging task. Direct screening of genomic libraries for highly expressed genes is an efficient way to identify promoters that confer high levels of gene expression. Tissue specificity of transgene expression is also an important consideration while deciding on the choice of the promoter, in order to increase the level of expression of the transgene. Thus, the strength of the promoter and the possibility of using stress inducible, developmental-stage- or tissue-specific promoters need to be considered (Bajaj *et al.*, 1999).

Besides, attention also needs to be focused on regulation of expression of plastid genes as well as to isolate target-specific promoters or design promoters with improved potential. Direct isolation of promoters can be done via T-DNA tagging with a promoterless reporter gene, although the most commonly used reporter gene for this kind of tagging has been the *gusA* gene (Casson *et al.*, 2002; Stangeland *et al.*, 2003), the ideal reporter gene should have a sensitive, nondestructive, and nontoxic assay allowing multiple *in vivo* screening rounds to identify simultaneously developmental specific, tissue specific, or stress-responsive patterns of expression.

The recent identification and isolation of a broad range of genes encoding different classes of proteins with activity against phytopathogenic fungi has opened the way to engineer fungus resistance into plants. Transgenic technology for imparting disease resistance requires tissue specific, wound- and pathogen-responsive promoters to express antifungal genes to control several diseases that threaten pigeonpea production. Similarly, for a number of future applications, transgenes will have to be expressed differentially or under specific abiotic (e.g., salt, wounding) stress conditions, which requires the use of a set of specific promoters to drive regulated gene expression. However, relatively few promoters are currently available for a specific or fine regulation of gene expression. It is expected that for these purposes homologous promoters will be more functional than heterologous ones, which should also raise less biosafety concerns.

3.3.5 Pyramiding of genes

Gene stacking is a term that is used in the context of genetically engineered crops, but is not a new idea in plant breeding. Gene stacking is combining desired traits into one line. Plant breeders are always stacking genes by making crosses between parents that each has a desired trait and then identifying offspring that have both of these desired traits. Pigeonpea breeders have been continually developing new varieties that contain the most effective combination of existing characters. A similar trend is expected with the pigeonpea transgenic plants by accumulation of

transgenes that inevitably becomes an increasing feature of new varieties. For example, stacking of different insecticidal genes might be considered as one of the major component of integrated pest management in pigeonpea. Bollgard® II developed by Monsanto that has been approved for commercialization in Australia and the United States in 2002 is an example for gene stacking, containing two *Bt* genes; *cry1Ac* and *cry2Ab2*. The proteins produced by these have different mode of action, thus making it very difficult for the pest to develop resistance to both the proteins simultaneously. Similarly, expected technological advancements in pigeonpea include identification and cloning of the genes responsible for traits such as high yield, disease resistance and tolerance to low temperature, to drought or to salt stresses. Although, this is a more difficult task because multiple genes control each trait for abiotic stress tolerance. However, in principle, the major genes involved in each of these traits can be identified first by mapping and then by map-based gene cloning (McCouch and Tanksley, 1991). This might ascertain that different varieties of transgenic pigeonpea plants endowed with a number of the above-mentioned desirable traits, will be grown in the dry and semi-arid regions of the world within the next 10 years.

3.4 Intellectual Property Rights (IPR) Issues

Intensive agriculture requires the use of certified seed (i.e., seed free of pathogens, pests, and weeds) and growers purchase new seed every year as an established practice. Historically, fertility and reproduction of grain and legume crops in Africa, Asia, and parts of the Americas have acquired a deep spiritual significance (Nuffiel Council on Bioethics, 1999). Nevertheless, the application of modern biotechnological techniques to plant species promises production of quality, quantity, and variety of food products. Hence, intellectual property rights are likely to play an important role in securing economic returns for the intellectual and financial investments that make the research and developments possible. The public-sector institutions need to obtain intellectual property rights for their discoveries so that these rights can be used in negotiations with the private sector to produce increased public benefit. Hence, intellectual property regimes might facilitate the development of beneficial new crop varieties through individual, public, and corporate sources, as well as promote research collaboration.

Most pigeonpea growers prefer plant hybrid varieties that are more uniform and vigorous than ordinary varieties because of heterosis (hybrid vigor) and these advantages are lost when second generation seed is used. However, in many instances, small growers cannot afford to purchase new seed every year, and they wish to maintain their long-standing practice of saving some of the seed from one year's crop in order to plant next year's crop. Although pigeonpea is one of the major grain legume crops grown in the tropics and subtropics, none of the pigeonpea transgenics have been commercialized so far. Therefore, intellectual property rights need to be narrowly tailored to be commensurate with the actual scope of new GM inventions so as not to impede continuing research, innovation, and development of this important pulse legume. IPR issues in pigeonpea needs to be focused on making GM pigeonpeas available to developing countries by establishing a partnership between the research institutions and industries so that the benefits of research, applications, and licensing become much more widely available. Moreover, broad patents should be granted to companies that secure their competitiveness in the market place (http://www.royalsoc.ac.uk/document.asp id=1447). Also, it is important to identify areas of common interest and opportunity between private sector and public-sector institutions so that the generation and use of pigeonpea transgenic not only to help resolve the intellectual property issues involved, but also benefit the poor.

4. CONCLUSIONS

Gene transfer techniques to develop transgenic crops can be seen as a logical extension of the crop plants for millennia displaying considerable potential to benefit both developed and developing countries. Genetic transformation is more expeditious, as the development of new cultivars by classical breeding typically takes from 10 to 15 years. Genetic transformation technology is critical in overcoming the severe bottlenecks associated with conventional

agricultural programs and enhancing their delivery prospects. However, transgenic technology needs to be used as adjunct to and not as substitutes for conventional technologies. Nevertheless, the primary attraction of the gene transfer methods to the plant breeder is the opportunity to tap into a wide gene pool to borrow traits, obviating the constraints of cross-compatible crop species. Most of the developments in plant gene transfer technology and the different strategies to produce improved transgenic plant varieties have been driven by the economic value of the species or the trait. These economic values, in turn, are mainly determined by their importance to agriculture in the developed world, particularly the United States and Western Europe. However, to increase global food production, it is necessary to ensure that this technology is effectively transferred to the developing world and adapted to local crops. The technological challenge here lies in obtaining improvements in agricultural productivity without destroying the global natural resource base. In the present scenario, many new approaches like gene tagging are being used to isolate resistance-conferring genes from resistant germplasm in crop species. Therefore, transgenic approaches can circumvent the difficulties of sterility and linkage drag, which do not allow the successful incorporation of resistance conferring genes from the wild species to crop species. Genetically engineered pigeonpea for virus resistance, insect resistance, and biofortification are good examples of strategies that could potentially benefit a diversity of legume crops. Substantial investments are therefore needed to develop, field test, and commercialize new transgenic pigeonpea varieties expressing insecticidal proteins, or proteins providing tolerance to herbicides or resistance to environmental stresses will revolutionize agriculture especially in arid and semi-arid regions of the world.

In addition, the scientific research aimed at risk analysis, prediction, and prevention, combined with adequate monitoring and stewardship, must continue so that negative ecological impact from GM crops will be kept to a minimum. One must also recognize the potential positive impact of GM crops on the environment, such as decreasing agricultural expansion to preserve wild ecosystems; improving air, soil, and water quality by promoting reduced tillage, reducing chemical and fuel use; improving biodiversity through resuscitation of older varieties and promotion of beneficial insects; and cleaning up contaminated soil and air through phytoremediation. Since the implications of a risk assessment of GM organisms are dependent on the social context, a participatory approach is needed to determine the balance of benefits to risks. By understanding the nature of genetic modifications and the nature of genomic plasticity in plants, it would be possible to determine an accepted safety baseline, against which the safety of the genetic engineering of plants can be evaluated. To ensure safe crops to humans and the environment, a strong, but not stifling, regulatory system needs to be established and properly implemented. A challenge for the future will be to use this technology wisely, as part of a long-term strategy to improve human health, preserve biodiversity, and promote more sustainable agricultural practices.

REFERENCES

Acland, J.D. (1971) *East African Crops: An Introduction to the Production of Field and Plantation Crops in Kenya, Tanzania and Uganda*. Longmans, London, pp. 140–141.

Adamczyk, J.J., Adamm, L.C. and Hardee, D.D. (2001a) Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *Journal of Economic Entomology* **94**, 1589–1593.

Adamczyk, J.J., Hardee, D.D., Adams, L.C. and Sumerford, D.V. (2001b) Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1Ac delta-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *Journal of Economic Entomology* **94**, 284–290.

Adu-Gyamfi, J.J., Fujita, K. and Ogata, S. (1990) Phosphorus fractions in relation to growth in pigeonpea (*Cajanus cajan* (L) Millsp.) at various levels of P supply. *Soil Science and Plant Nutrition* **36**, 531–543.

Ae, N., Arihara, J., Okada, K., Yoshihara, T. and Johansen, C. (1990) Phosphorus uptake by pigeonpea and its role in cropping system of the Indian subcontinent. *Science* **248**, 477–480.

Agarwal, R.R., Yadav, J.S.P. and Gupta, R.N. (1976) *Saline and Alkali Soils of India*. Indian Council for Agricultural Research, New Delhi.

Ahlawat, I.P.S., Gangaiah, B. and Singh, I.P. (2005) Pigeonpea (*Cajanus cajan*) research in India—an overview. *Indian Journal of Agricultural Science* **75**, 309–320.

AICPIP (All India Coordinated Pigeonpea Improvement Project) (1999) Annual report of AICPIP. Indian Institute of Pulses Research, Indian Council of Agricultural Research (ICAR), Kanpur, Uttar Pradesh, India.

Aiyer, A.K.Y.N. (1949) Mixed cropping in India. *Indian Journal of Agricultural Science* **19**, 439–543.

Al-Nahidi, S., Saxena, K.B., Ward, C.S., Mehdi Bushariy, A. and Kumar, R.V. (2001) Evaluation of pigeonpea cultivars through farmers' participation in Yemen. *Afro-Asian Journal of Rural Development* **34**, 63–70.

Amtsblatt Der Europäischen Gemeinschaften (1998) Stellungnahme des Wirtschafts-und Sozialausschusses zum Thema "Antibiotika-Resistenz-eine Bedrohung der Volksgesundheit" 98/C 407/02.

Apse, M.P., Aharon, G.S., Snedden, W.A. and Blumwald, E. (1999) Salt tolerance conferred by overexpression of vacuolar Na+/H +antiport in *Arabidopsis*. *Science* **285**, 1256–1258.

Ariyanayagam, R.P., Nageshwar, A. and Zaveri, P.P. (1993) Cytoplasmic-genic male sterility in interspecific matings of *Cajanus* crop. *Crop Science* **35**, 981–985.

Ariyanayagam, R.P. and Spence, J.A. (1978) A possible gene spurce for early, day neutral pigeonpeas, *Cajanus cajan* (L.) Millsp. *Euphytica* **27**, 505–509.

Arundhati, A. (1999) *Agrobacterium*-mediated transformation of pigeonpea (*Cajanus cajan* L. Millsp.) by using leaf disks. *International Chickpea and Pigeonpea Newsletter* **6**, 62–64.

Bajaj, S., Targolli, J., Liu, L.-F., Ho, T.H. and Wu, R. (1999) Transgenic approaches to increase dehydration stress tolerance in plants. *Molecular Breeding* **5**, 493–503.

Bajaj, Y.P.S. (ed.) (1990) *Biotechnology in Agriculture and Forestry*. Springer-Verlag, Berlin, pp. 3–37.

Barton, K., Whitely, H. and Yang, N.S. (1987) *Bacillus thuringiensis* δ-endotoxin in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiology* **85**, 1103–1109.

Bent, A.F. and Yu, I.C. (1999) Applications of molecular biology to plant disease and insect resistance. *Advances in Agronomy* **66**, 251–298.

Bentham, G. (1837) *Commentaiones de Leguminosarum Generibus*. Sollingeri, Vienna.

Bentham, G., and Hooker, J.D. (1865) *Genera Plantarum*. Reeve and Co., London.

Bevan, M., Barnes, W.M. and Chilton, M.D. (1983) A Chimeric antibiotic resistance gene as a selectable marker for plant cell transformation. *Nature* **304**, 184–187.

Birch, R.G. (1997) Plant transformation: problems and strategies for practical application. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 297–326.

Bryant, J. and Leather, S. (1992) Removal of selectable marker genes from transgenic plants: needless sophistication or social necessity. *Trends in Biotechnology* **10**, 274–275.

Campbell, B.T., Baenziger, P.S., Mitra, A., Sato, S. and Clemente, T. (2000) Inheritance of multiple transgenes in wheat. *Crop Science* **40**, 1133–1141.

Casson, S.A., Chilley, P.M., Topping, J.F., Evans, I.M., Souter, M.A. and Lindsey, K. (2002) The *POLARIS* gene of *Arabidopsis* encodes a predicted peptide required for correct root growth and leaf vascular patterning. *Plant Cell* **14**, 1705–1721.

Chauhan, Y.S. (1987) Screening for tolerance to salinity and water logging: case studies with pigeonpea and chickpea. In: *Adaptation of Chickpea and Pigeonpea to Abiotic Stresses. Proceedings of the Consultant's Workshop*. December 19–21, 1984 ICRISAT Center, India. ICRISAT, Patancheru, AP, India, pp. 99–103.

Crouch, J.H. and Ortiz, R. (2004) Applied genomics in the improvement of crops grown in Africa. *African Journal of Biotechnology* **3**, 489–496.

Dale, P.J. (1992) Spread of engineered genes to wild relatives. *Plant Physiology* **100,** 13–15.

Daniell, H. and Khan, M.S. (2003) Engineering the chloroplast genome for biotechnology applications. In: Neal, C.N. (ed.) *Transgenic Plants: Current Innovations and Future Trends*. Horizon Scientific Publications, Wymondham, Norfolk, pp. 83–111.

Davey, M.R., Kumar, V. and Hammatt, N (1994) In vitro cultures of legumes. In: Vasil, I.K. and Thorpe, T.A. (eds.) *Plant Cell and Tissue Culture*. Kluwer, Dordrecht, pp. 313–329.

Davies, G.J., Kilby, J., Riou-Khamlichi, C. and Murray, J.A.H. (1999) Somatic and germinal inheritance of an FLP-mediated deletion in transgenic tobacco. *Journal of Experimental Botany* **50**, 1447–1456.

Dayal, S., Lavanya, M., Devi, P. and Sharma, K.K. (2003) An efficient protocol; for shoot regeneration and genetic transformation of pigeonpea (*Cajanus cajan* (L) Millsp.) using leaf explants. *Plant Cell Reports* **21**, 1072–1079.

Deodikar, L. and Thakur, R. (1956) Genetics and improvement of the pigeonpea (*Cajanus indicus* Spreng). *Ceres Minas Gerais* **10**, 20–44.

Dodia, D.A., Patel, A.J., Patel, I.S., Dhulia, F.K. and Tikka, S.B.S. (1996) Antibiotic effect of pigeonpea wild relatives on *Helicoverpa armigera*. *International Chickpea and Pigeonpea Newsletter* **3**, 100–101.

Duke, J.A. (1981) *Handbook of Legumes of World Economic Importance*. Plenum Press, New York, pp. 33–37.

Evans, R., Wang, A., Hanten, J., Altendorf, P. and Mettler, I. (1996) A positive selection system for maize transformation. *In Vitro Cellular and Developmental Biology—Plant* **32**, 72A.

FAOSTAT website (2005) http://faostat.fao.org/faostat/collections?version=ext&hasbulk=0&subset=agriculture.

Faris, D.G., Saxena, K.B., Mazumdar, S. and Singh, U. (1987) *Vegetable Pigeonpea: A Promising Crop for India*. ICRISAT, Patancheru, p. 13.

Fischhoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., MvCormick, S.M., Niedermeyer, J.G., Dean, D.A., Kusano-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rogers, S.G. and Fraley, R.T. (1987) Insect tolerant tomato plants. *Bio/Technology* **5**, 807–812.

Fontanna, G.S., Santini, L., Caretto, S., Frugis, G. and Mariotti, D. (1993) Genetic transformation in the grain legume *Cicer arietinum* L. (chickpea). *Plant Cell Reports* **12**, 194–198.

Fujita, K., Kai, Y., Takayanagi, M., El-Shemy, H., Adu-Gyamfi, J.J. and Mohapatra, P.K. (2004) Genotypic variability of pigeonpea in distribution of photosynthetic carbon at low phosphorus level. *Plant Science* **166**, 641–649.

Gaxiola, R.A., Li, J., Unurraga, S., Dang, L.M., Allen, G.J., Alper, S.L. and Fink, G.R. (2001) Drought- and salt-tolerant plants result form overexpression of the AVP1 H+−pump. *Proceedings of the National Academy of Sciences of the USA* **98**, 11444–11449.

Geetha, N., Venkatachalam, P. and Laxmi Sita, G. (1999) *Agrobacterium*-mediated genetic transformation of pigeonpea (*Cajanus cajan L.*) and development of transgenic plants via direct organogenesis. *Plant Biotechnology* **16**, 213–218.

Geetha, N., Venkatachalam, P., Prakash, V. and Laxmi Sita, G. (1998) High frequency induction of multiple shoots and plant regeneration from seedling explants of pigeonpea (*Cajanus cajan* (L) Millsp.). *Current Science* **10**, 1036–1041.

George, L. and Eapen, S. (1994) Organogenesis and embryogenesis from diverse explants in pigeonpea *(Cajanus cajan L.*). *Plant Cell Reports* **13**, 417–420.

Gidoni, D., Bar, M., Leshem, B., Gilboa, N., Mett, A. and Feiler, J. (2001) Embryonal recombination and germline inheritance of recombined *FRT* loci mediated by constitutively expressed FLP in tobacco. *Euphytica* **121**, 145–156.

Gleave, A.P., Mitra, D.S., Mudge, S.R. and Morris, B.A.M. (1999) Selectable maker-free transgenic plants without sexual crossing: transient expression of cre recombinase and use of a conditional lethal dominant gene. *Plant Molecular Biology* **40**, 223–235.

Grant, J.E. (1990) Soybean: wide hybridization through embryo culture. In: Bajaj, Y.P.S. (ed.) *Biotechnology in Agriculture and Forestry*. Springer-Verlag, Berlin, Vol. **10**, pp. 134–148.

Greilhuber, J. and Obermayer, R. (1998) Genome size variation in *Cajanus cajan* (Fabaceae): a reconsideration. *Plant Systematics and Evolution* **212**, 135–141.

Gressel, J. (1992) Indiscriminate use of selectable markers—sowing wild oats? *Trends in Biotechnology* **10**, 382.

Grover, A., Agarwal, M., Katiyar-Agarwal, S., Sahi, C. and Agarwal, S. (2000) Production of high temperature tolerant transgenic plants through manipulation of membrane lipids. *Current Science* **79**, 557–559.

Haldrup, A., Petersen, S.G. and Okkels, F.T. (1998a) The xylose isomerase gene from *Thermoanaerobacterium thermosulfurogenes* allows effective selection of transgenic plant cells using D-xylose as the selection agent. *Plant Molecular Biology* **37**, 287–296.

Haldrup, A., Petersen, S.G. and Okkels, F.T. (1998b) Positive selection: a plant selection principle based on xylose isomerase, an enzyme used in the food industry. *Plant Cell Reports* **18**, 76–81.

Harvey, A., Moisan, L., Lindup, S. and Lonsdale, D. (1999) Wheat regenerated from scutellum callus as a source of material for transformation. *Plant Cell, Tissue and Organ Culture* **57**, 153–156.

Herrera-Estrella, L., Deblock, M., Messens, E., Hernalsteens, J.P., Van Montagu, M. and Schell, J. (1983) Chimeric genes as dominant selectable markers in plants cells. *European Molecular Biology Organisation Journal* **2**, 987–995.

Hilder, V.A. and Boulter, D. (1999) Genetic engineering of crop plants for insect resistance a critical review. *Crop Protection* **18**, 177–191.

Howard, A., Howard, G.L.C. and Khan, A.R. (1919) Studies in the pollination of Indian crops. I. Memoris. *Department of Agriculture, India (Botanical series)* **10**, 195–200.

ICRISAT (1992) *The Medium Term Plan*. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru, Vol. 1, p. 80.

Jaiwal, P.K., Christof, S. and Potrykus, I. (1998) *Agrobacterium rhizogenes*-mediated gene transfer in mungbean (*Vigna radiata*). *Current Science* **75**, 41–45.

Jaiwal, P.K., Sahoo, L., Singh, N.D. and Singh, R.P. (2002) Strategies to deal with the concern about marker genes in transgenic plants: some environment friendly approaches. *Current Science* **83**, 128–136.

James, C. (2002) Preview: global status of commercialized transgenic crops. ISAAA Briefs No. 27, ISAAA, Ithaca, NY, USA.

James, C. (2005) Preview: global status of commercialized biotech/GM Crops. ISAAA Briefs No. 32, ISAAA, Ithaca, NY, USA.

Jia, H., Pang, Y., Chen, X. and Fang, R. (2006) Removal of the selectable marker gene from transgenic tobacco plants by expression of *Cre* recombinase from a tobacco mosaic virus vector through agroinfection. *Transgenic Research* **15**, 375–384.

Joersbo, M., Donaldson, I., Kreiberg, J., Petersen, S.G., Brunstedt, J. and Okkels, F.T. (1998) Analysis of mannose selection used for transformation of sugar beet. *Molecular Breeding* **4**, 111–117.

Joersbo, M., Mikkelsen, J.D. and Brunstedt, J. (2000) Relationship between promoter strength and transformation frequencies using mannose selection for the production of transgenic sugar beet. *Molecular Breeding* **6**, 207–213.

Joersbo, M., Petersen, S.G. and Okkels, S.G. (1999) Parameters interacting with mannose selection employed for the production of transgenic sugar beet. *Physiologia Plantarum* **105**, 109–115.

Jones, T.A., Lava Kumar, P., Saxena, K.B., Kulkarni, N.K., Muniyappa, V. and Waliyar, F. (2004) Sterility mosaic disease—the "Green Plague" of pigeonpea. *Plant Disease* **88**, 436–445.

Jordan, M.C. (2000) Green fluorescent protein as a visual marker for wheat transformation. *Plant Cell Reports* **19**, 1069–1075.

Kannaiyan, J., Nene, Y.L., Reddy, M.V., Ryan, J.G. and Raju, T.N. (1984) Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. *Tropical Pest Management* **30**, 62–71.

Kay, D.E. (1979) Food legumes. TPI Crop and Product Digest No. 3, London, UK, pp. 26–47.

Keating, B.A. and Fisher, M.J. (1985) Comparative tolerance of tropical grain legumes to salinity. *Australian Journal of Agricultural Sciences* **36**, 373–383.

Keen, N.T. (1999) Plant disease resistance: progress in basic understanding and practical application. *Advances in Botanical Research* **30**, 292–328.

Kilby, N.J., Davies, G.J., Snaith, M.R. and Murray, J.A.H. (1995) FLP recombinase in transgenic plants: constitutive activity in stably transformed tobacco and generation of marked cell clones in *Arabidopsis*. *Plant Journal* **8**, 637–652.

Komari, T., Hiei, Y., Saito, Y., Murai, N. and Kumashiro, T. (1996) Vectors carrying two separate T-DNAs for co-transformation of higher plants mediated by *Agrobacterium tumefaciens* and segregation of transformants free from selection markers. *Plant Journal* **10**, 165–174.

Krattiger, A.F. (1997) Insect resistance in crops: a case study of bacillus thuringiensis and its transfer to developing countries. International Society for the Acquisition of

Agrobiotech Applications (ISAAA) Briefs No. 2, ISAAA, Ithaca, NY, USA.

Krishnamurthy, K.V, Suhasini, K., Sagare, A.P., Meixner, M., de Kathen, A., Pickardt, T. and Schieder, O. (2000) *Agrobacterium* mediated transformation of chickpea (*Cicer arietinum* L.) embryo axes. *Plant Cell Reports* **19**, 235–240.

Kumar, A.S., Reddy, T.P. and Reddy, G.M. (1983) Plantlet regeneration from different callus cultures of pigeonpea. *Plant Science Letters* **32**, 271–278.

Kumar, A.S., Reddy, T.P. and Reddy, G.M. (1984) Multiple shoots from cultured explants of pigeonpea and *Atylosia* species. *SABRAO Journal* **16**, 101–105.

Kumar, A.S., Reddy, T.P. and Reddy, G.M. (1985) Genetic analysis of certain in vitro and in vivo parameters in pigeonpea (*Cajanus cajan* L.). *Theoretical and Applied Genetics* **70**, 151–156.

Kumar, S.M., Sharma, K.K. and Devi, P. (2004b) Transformation of pigeonpea with rice chitinase gene. *Plant Breeding* **123**, 485–489.

Kumar, S.M., Syamala, D., Sharma, K.K. and Devi, P. (2004a) *Agrobacterium tumefaciens* mediated genetic transformation of pigeonpea (*Cajanus cajan* (L.) Millsp.). *Journal of Plant Biotechnology* **6**, 69–75.

Kumar Rao, J.V.D.K., Thompson, J.A., Sastry, P.V.S.S., Giller, K.E. and Day, J.M. (1987) Measurement of N_2-fixation in field-grown pigeonpea [*Cajanus cajan* (L.) Millsp.] using 15N-labelled fertilizer. *Plant and Soil* **101**, 107–113.

Lawrence, P.K. and Koundal, K.R. (2001) *Agrobacterium tumefaciens* mediated transformation of pigeonpea (*Cajanus cajan* L. Millsp.) and molecular analysis of regenerated plants. *Current Science* **80**, 1428–1432.

Levin, M.A. and Strauss, H. (1993) Overview of risk assessment. In: Levin, M.A. and Strauss, H. (eds.) *Risk Assessment in Genetic Engineering*. McGraw Hill, New York, pp. 1–17.

Levy, S.B. (1997) Antibiotic resistance: an ecological imbalance. In: Chadwick, D. and Goode, J. (eds.) *Antibiotic Resistance: Origins, Evolution, Selection and Spread. Ciba Foundation Symposium 207*. John Wiley & Sons, Chichester, pp. 1–14.

Luo, H., Lyznik, L.A., Gidoni, D. and Hodges, T.K. (2000) FLP-mediated recombination for use in hybrid plant production. *Plant Journal* **23**, 423–430.

Lyznik, L.A., Rao, K.V. and Hodges, T.K. (1996) FLP-mediated recombination of FRT sites in the maize genome. *Nucleic Acids Research* **24**, 3784–3789.

Mallikarjuna, N., Jadhav, D. and Reddy, P. (2006) Introgression of *Cajanus platycarpus* genome into cultivated pigeonpea, *C. cajan*. *Euphytioca* **149**, 161–167.

Marley, P.S. and Hillocks, R.J. (1996) Effect of root-knot nematodes (*Meloidogyne* spp.) on *Fusarium* wilt in pigeonpea (*Cajanus cajan*). *Field Crops Research* **46**, 15–20.

Matzke, M.A. and Matzke, A.J.M. (1991) Differential inactivation and methylation of a transgene in plants by two suppressor loci containing homologous sequences. *Plant Molecular Biology* **16,** 821–830.

Matthews, P.R., Gubler, F. and Jacobsen, J.V. (1997) A plant based expression system for matching cDNA clones and isozymes. *Plant Molecular Biology Reporter* **15**, 163–169.

McCormac, A.C., Fowler, M.R., Chen, D.F. and Elliott, M.C. (2001) Efficient co-transformation of *Nicotiana tabacum* by two independent T-DNAs, the effect of T-DNA size and implications for genetic separation. *Transgenic Research* **10**, 143–155.

McCouch, S.R. and Tanksley, S.D. (1991) Development and use of restriction fragment length polymorphism in rice breeding and genetics. In: *Rice Biotechnology*. International Rice Research Institute, Manila, pp. 109–133.

Mehta, U. and Mohan Ram, H.Y. (1980) Regeneration of plantlets from cotyledons of *Cajanus cajan*. *Indian Journal of Experimental Biology* **18**, 800–802.

Miller, M., Tagliani, L., Wang, N., Berka, B. and Bidney, D. (2002) High efficiency transgene segregation in co-transformed maize plants using an *Agrobacterium tumefaciens* 2 T-DNA binary system. *Transgenic Research* **11**, 381–396.

Mohan, M.L. and Krishnamurthy, K.V. (1998) Plant regeneration in pigeonpea (*Cajanus cajan* (L) Millsp.) by organogensis. *Plant Cell Reports* **17**, 705–710.

Monnier, M. (1990) Plant cell and tissue culture. In: Pollard, J.W. and Walker, J.M. (eds.) *Methods in Molecular Biology*. Humana Press, Clifton, pp. 129–139.

Morton, J.F. (1976) The pigeonpea (*Cajanus cajan* Millsp.), a high protein tropical bush legume. *HortScience* **11**, 11–19.

Mouras, A., Suharsono, S., Hernould, M., Zabaleta, E. and Araya, A. (1999) Inheritance of an induced male-sterile trait in transgenic plants expressing an engineered unedited *atp9* mitochondrial gene. *Theoretical and Applied Genetics* **98**, 614–621.

Muller, R.A.E., Parthasarathy Rao, P. and Subba Rao, K.V. (1990) Pigeonpea: markets and outlook. In: Nene, Y.L., Hall, S.D. and Sheila, V.K. (eds.) *The Pigeonpea*. CAB International, Wallingford, pp. 457–479.

Murugarajendran, C., Ali Kahn, W.M., Rathnaswamy, R., Rangaswamy, S.R.R., Rai Kesavan, R., Kalaimagal, T., Saxena, K.B. and Kumar, R.V. (1995) IPH 732—a new hybrid pigeonpea for Tamil Nadu. *International Chickpea and Pigeonpea Newsletter* **2**, 55–57.

Muthukumar, B., Mariamma, M., Veluthambi, K. and Gnanam, A. (1996) Genetic transformation of cotyledon explants of cowpea (*Vigna unguiculata* L. Walp) using *Agrobacterium tumefaciens*. *Plant Cell Reports* **15,** 980–985.

Nayak, P., Basu, D., Das, S., Basu, A., Ghosh, D., Ramakrishnan, N.A., Ghosh, M. and Sen, S.K. (1997) Transgenic elite indica rice plants expressing CryIAc deltaendotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). *Proceedings of the National Academy of Sciences of the USA* **94**, 2111–2116.

Negrotto, D., Jolley, M., Beer, S., Wenck, A.R. and Hansen, G. (2000) The use of phosphomannose isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. *Plant Cell Reports* **19**, 798–803.

Nuffiel Council on Bioethics (1999) The use of genetically modified crops in developing countries: a follow up discussion paper. London, UK. www.nuffieldbioethics.org/gmcrops.

O'Callaghan, M., Glare, T.R., Burgess, E.P.J. and Malone, L.A. (2005) Effect of plants genetically modified for insect resistance on non target organisms. *Annual Review of Entomology* **50**, 271–292.

Okiror, M.A. (1986) Breeding for resistance to Fusarium wilt. Ph.D. Thesis, University of Nairobi, Kenya.

Ong, C.K. and Daniel, J.N. (1990) Le pois d'Angole. *L'Agroforesterie Aujourdh'hui* **2**, 4–7.

Onouchi, H., Yokoi, K., Machida, C., Matsuzaki, H., Oshima, Y., Matsuoka, K., Nakamura, K. and Machida, Y. (1991) Operation of an efficient site-specific recombination system of *Zygosaccharomyces rouxii* in tobacco cells. *Nucleic Acids Research* **19**, 6373–6378.

Ortiz, R. (1998) Critical role of plant biotechnology for the genetic improvement of food crops—perspective for the next millenuim. *Electronic Journal of Biotechnology.* http://www.ejbiotechnology.info/content/vol1/issue3/full/7/.

Osiru, D.S.O. and Kibira, G.R. (1981) Sorghum/pigeonpea and finger millet/groundnut mixtures with special reference to plant population and crop arrangement. *Proceedings of the International Workshop on Intercropping*. January 10–13, 1979 ICRISAT, Patancheru, AP, India, pp. 78–85.

Patel, D.B., Brave, D.M., Nagar, N. and Mehta, A.R. (1994) Regeneration of pigeonpea, *Cajanus cajan*, through somatic embryogenesis. *Indian Journal of Experimental Biology* **32**, 740–744.

Patnaik, D. and Khurana, P. (2001) Wheat biotechnology: a minireview. *Electronic Journal of Biotechnology* **4**, 75–102.

Prasad, V., Sanjaya Satyavathi, V.V., Valli, K.M., Khandelwal, A., Shaila, M.S. and Lakshmi Sita, G. (2004) Expression of biologically active hemagglutinin-neuraminidase protein of *Peste des petits ruminants* virus in transgenic pigeonpea (*Cajanus cajan* (L) Millsp.). *Plant Science* **166**, 199–205.

Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T. J. V. and Hogan, S.P. (2005) Transgenic expression of bean α-amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agricultural and Food Chemistry* **53**, 9023–9030.

Pundir, R.P.S. and Singh, R.B. (1987) Possibility of genetic improvement of pigeonpea (*Cajanus cajan* (L.) Millsp.) utilizing wild gene sources. *Euphytica* **36**, 33–37.

Purseglove, J.W. (ed.) (1968) *Tropical Crops-Dicotyledons*. Longmans, London, pp. 236–237.

Purseglove, J.W. (ed.) (1988) *Tropical Crops-Dicotyledons, ELBS edition*. Longmans, London.

Rai, B. (1979) *Heterosis Breeding*. Agro-Biological Publication, Delhi.

Ranjekar, P.K., Patankar, A., Gupta, V., Bhatnagar, R., Bentur, J. and Kumar, P.A. (2003) Genetic engineering of crop plants for insect resistance. *Current Science* **84**, 321–329.

Reddy, B.V.S., Green, J.M. and Bisen, S.S. (1978) Genetic male-sterility in pigeonpea. *Crop Science* **18**, 362–364.

Reddy, M.V., Sharma, S.B. and Nene, Y.L. (1990) Pigeonpea: disease management. In: Nene, Y.L., Hall, S.D. and Sheila, V.K. (eds.) *The Pigeonpea*. Common Wealth Agricultural Bureaue International, Wallingford, pp. 303–347.

Rego, T.J. and Nageswara Rao, V. (2000) Long-term effects of grain legumes on rainyseason sorghum productivity in a semi-arid tropical vertisol. *Experimental Agriculture* **36**, 205–221.

Roa-Rodriguez, C. and Nottenburg, C. (2003) *Antibiotic Resistance Genes and Their Uses in Genetic Transformation*. CAMBIA IP Resource, Canberra. http://www.cambiaip.org.

Rommens, C.M. and Kishmore, G.M. (2000) Exploiting the full potential of disease-resistance genes for agricutural use. *Current Opinion in Biotechnology* **11**, 120–125.

Roy, A. and De, D.N. (1967) Intergeneric hybridization of *Cajanus* and *Atylosia*. *Science and Culture* **31**, 93–95.

Ruiz, O.N. and Daniell, H. (2005) Engineering cytoplasmic male sterility via the chloroplast genome. *Plant Physiology* **138**, 1232–1246.

Sanyal, I., Singh, A.K., Kaushik, M. and Amla, D.V. (2005) *Agrobacterium*-mediated transformation of chickpea (*Cicer arietinum* L.) with *Bacillus thuringiensis cry1Ac* gene for resistance against pod borer insect *Helicoverpa armigera*. *Plant Science* **168**, 1135–1146.

Satyavathi, V.V., Prasad, V., Khandelawl, A., Shaila, M.S. and Lakshmi Sita, G. (2003) Expression of hemagglutinin protein of rinderpest virus in transgenic pigeonpea (*Cajanus cajan* (L.) Millsp.) plants. *Plant Cell Reports* **21** 651–658.

Saxena, K.B. (2004) Prospects of commercial exploitation of hybrid vigor in legumes—a success story of pigeonpea. *Proceedings of the 5th European Conference on Grain Legumes. Legumes for the benefit of agriculture nutrition and the environment; their genomics, their products, and their environment*. Dijon, France, p. 112.

Saxena, K.B., Chauhan, Y.S., Kumar, R.V., Ariyanayagam, R.P., Srivastava, D.P., Johansen, C. and Asthana, A.N. (1997) Heterosis breeding for higher yield in pigeonpea. In: Asthane, A.N. and Ali, M. (eds.) *Recent Advances in Pulses Research*. IIPR, Kanpur, pp. 109–125.

Saxena, K.B. and Kumar, R.V. (2003) Development of a cytoplasmic nuclear male sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. *Indian Journal of Genetics and Plant Breeding* **63**, 225–229.

Saxena, K.B., Kumar, R.V. and Rao, P.V. (2002) Pigeonpea nutrition and its improvement. In: Basra, A.S. and Randhawa, I.S. (eds.) *Quality Improvement in Field Crops*. Food Products Press-The Haworth Press., Binghamton, pp. 227–260.

Saxena, K.B., Rao, A.N., Singh, U. and Remanandan, P. (1996) Intraspecies variation in *Cajanus platycarpus* for some agronomic traits and crossability. *International Chickpea and Pigeonpea Newsletter* **3**, 49–51.

Saxena, K.B., Singh, L. and Gupta, M.D. (1990) Variation for natural outcrossing in pigeonpea. *Euphytica* **46**, 143–148.

Saxena, K.B., Srivastava, D.P., Chauhan, Y.S. and Ali, M. (2005) Hybrid pigeonpea. In: Ali, M. and Kumar, S. (eds.) *Advances in Pigeonpea Research*. Indian Institute of Pulses Research, Kanpur, pp. 96–133.

Saxena, K.B., Wallis, E.S. and Byth, D.E. (1983) A new gene for male sterility in pigeonpea. *Euphytica* **39**, 143–148.

Schuler, T.H., Poppy, G.M., Kerry, B.R. and Denholm, I. (1998) Insect resistant transgenic plants. *Trends in Biotechnology* **16**, 168–175.

Sharma, H.C., Sharma, K.K. and Crouch, J.H. (2004) Genetic transformation of crops for insect resistance: potential and limitations. *Critical Reviews in Plant Sciences* **23**, 47–72.

Sharma, H.C., Sharma, K.K., Seetharama, N. and Oritz, R. (2001) Genetic transformation of crop plants: risks and opportunities for the rural poor. *Current Science* **80**, 1495–1508.

Sharma, K.K., Sharma, H.C., Seetharama, N. and Ortiz, R. (2002) Development and deployment of transgenic plants: biosafety considerations. *In Vitro Cellular and Developmental Biology—Plant* **38**, 106–115.

Sharma, K.K., Bhatnagar-Mathur, P. and Thorpe, T.A. (2005) Genetic transformation technology: status and problems. *In Vitro Cellular and Developmental Biology—Plant* **41**, 102–112.

Sharma, K.K., Lavanya, M. and Anjaiah, V. (2006a) *Agrobacterium*-mediated production of transgenic pigeonpea (*Cajanus cajan L. Millsp.*) expressing the synthetic BT *cry1Ab* gene. *In Vitro Cellular and Developmental Biology - Plant* **42**, 165–173.

Sharma, K.K., Sreelatha, G. and Dayal, S. (2006b) Pigeonpea [*Cajanus cajan* L. (Millsp.)]. In: Wang, K. (ed.) *Agrobacterium Protocols. Methods in Molecular Biology*. Humana Press, Totowa, Vol. **44**, pp. 359–367.

Sheldrake, A.R. (1984) Pigeonpea. In: Goldsworthy, P.R. and Fisher, N.M. (eds.) *The Physiology of Tropical Field Crops*. John Wiley & Sons, Chichester.

Shi, H., Lee, B.H., Wu, S.J. and Zhu, J.K. (2003) Over expression of a plasma membrane Na+/H+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. *Nature Biotechnology* **21**, 81–85.

Shivaparakash, N., Pental, D. and Bhalla-Sarin, N. (1994) Regeneration in pigeonpea (*Cajanus cajan* (L) Millsp.) from cotyledonary node via multiple shoot formation. *Plant Cell Reports* **13**, 623–627.

Singh, L., Sharma, D. and Deodhar, A.D. (1974) Effect of environments on the protein of seeds and implication in pulse improvement. *Indian Journal of Genetics and Plant Breeding* **34**, 764–808.

Singh, N.D., Sahoo, L., Saini, R., Sarin, N.B. and Jaiwal, P.K. (2004) *In vitro* regeneration and recovery of primary transformants from shoot apices of pigeonpea using *Agrobacterium tumefaciens*. *Physiology and Molecular Biology of Plants* **10**, 65–74.

Singh, N.D., Sahoo, L., Sonia and Jaiwal, P.K. (2002) *In vitro* shoot organogenesis and plant regeneration from cotyledonary node and leaf explants of pigeonpea (*Cajanus cajan* L. Millsp). *Physiology and Molecular Biology of Plants* **8**, 133–140.

Solomon, S., Argikar, G.P., Solanki, M.S. and Morbad, I.R. (1957) A study of heterosis in *cajanus cajan* (L.) Millsp. *Indian Journal of Genetics and Plant Breeding* **17**, 90–95.

Souframanien, J., Manjaya, J.G., Krishna, T.G. and Pawar, S.E. (2003) Random amplified polymorphic DNA analyses of cytoplasmic male sterile and male fertile pigeonpea (*Cajanus cajan* (L.) Millsp.). *Euphytica* **129**, 293–299.

Sreelatha, G. (2006) Genetic transformation for pod borer resistance through agrobacterium in pigeonpea (*Cajanus cajan* (L.)). Ph.D. Thesis, Osmania University, Hyderabad, India.

Stangeland, B., Salehian, Z., Aalen, R., Mandal, A. and Olsen, O.A. (2003) Isolation of GUS marker lines for genes expressed in *Arabidopsis* endosperm, embryo, and maternal tissues. *Journal of Experimental Botany* **54**, 279–290.

Stewart Jr, C.N., Adang, M.J., All, J.N., Boerma, H.R, Cardineau, G., Tucher, D. and Parrott, W.A. (1996) Genetic transformation, recovery, and characterization of fertile soybean transgenic for a synthetic *Bacillus thuringiensis cryIAc* gene. *Plant Physiology* **112**, 121–129.

Stewart Jr, C.N., Halfhill, M.D. and Warkwick, S.I. (2003) Transgene introgression from genetically modified crops to their wild relatives. *Nature Review of Genetics* **4**, 806–817.

Subbarao, G.V. (1988) Salinity tolerance in pigeonpea (*Cajanus cajan*) and its wild relatives. Ph.D. Dissertation, Indian Institute of Technology, Kharagpur, India.

Sugita, K., Kasahara, T., Matsunaga, E. and Ebinuma, H. (2000) A transformation vector for the production of marker-free transgenic plants containing a single copy transgene at high frequency. *Plant Journal* **22**, 461–469.

Sugita, K., Matsunaga, E. and Ebinuma, H. (1999) Effective selection for generating marker-free transgenic plants independent ulated expression of plant genes. *Plant Cell Reports* **18**, 941–947.

Surekha, C.H., Beena, M.R., Arundhati, A., Singh, P.K., Tuli, R., Dutta-Gupta, A. and Kirti, P.B. (2005) *Agrobacterium*-mediated genetic transformation of pigeonpea (*Cajanus cajan* (L.) Millsp.) using embryonal segments and development of transgenic plants for resistance against Spodoptera. *Plant Science* **169**, 1074–1080.

Tanida, M. (1996) Catalase activity of rice seed embryo and its relation to germination rate at a low temperature. *Breeding Science* **46**, 23–27.

Tanida, M. and Saruyama, H. (1995) Catalase activity in embryos and its relation to germination ability of rice and wheat seeds. In: Noda, K. and Mares, D.J. (eds.) *Seventh International Symposium on Pre-Harvest Sprouting in Cereals*. Center for Academic Societies of Japan, Osaka, pp. 357–361.

Thu, T.T., Mai, T.T.X., Deaele, E., Faris, S., Tadesse, Y., Angenon, G. and Jacobs, M. (2003) *In vitro* regeneration and transformation of pigeonpea (*Cajanus cajan* (L) Millsp). *Molecular Breeding* **11**, 159–168.

Tikka, S.B.S., Parmar, L.D. and Chauhan, R.M. (1997) First record of cytoplasmic-genic male-sterility system in pigeonpea (*Cajanus cajan* (L.) Millsp.) through wide hybridization. *Gujarat Agricultural University Research Journal* **22**, 160–162.

Troedson, R.J., Wallis, E.S. and Singh, L. (1990) Pigeonpea: adaptation. In: Nene, Y., Hall, S.D. and Sheila, V.K. (eds.) *The Pigeonpea*. CAB International, Wallingford, pp. 159–177.

Vaeck, M., Reynaerts, A., Hoftey, H., Jansens, S., DeBeuckleer, M., Dean, C., Zabeau, M., Van Montagu, M. and Leemans, J. (1987) Transgenic plants protected from insect attack. *Nature* **327**, 33–37.

Van Den Elzen, P.J.M., Townseed, J., Lec, K.Y. and Bedbrook, J.R. (1985) A chimeric hygromycin resistance gene as a selectable marker in plant cells. *Plant Molecular Biology* **5**, 299–302.

Van Der Maesen, L.J.G. (1983) World distribution of pigeonpea. ICRISAT Information Bulletin No. 14., ICRISAT, Patancheru, AP, India, p. 40.

Van Der Maesen, L.J.G. (1986) *Cajanus DC. and Athylosia W. and A. (Leguminosae)*. Wageningon Papers, Agricultural University, Wageningon, pp. 225.

Velkov, V.V. (2001) Stress-induced evolution and biosafety of genetically modified microorganisms released into the environments. *Journal of Biosciences* **26**, 667–683.

Velkov, V.V., Sokolov, M.S. and Medvinsky, A.B. (2003a) The problems of the state regulation of production of transgenic plants. (In Russian). *Vestnik Zashchity Rastenii (Plant Protection News)* **33**, 16.

Velkov, V.V., Sokolov, M.S. and Medvinskym, A.B. (2003b) Assessment of the ecological risks of production of the transgenic insecticidal plants (In Russian). *Agrokhimiya (Agricultural Chemistry)* **2**, 74–96.

Vergunst, A.C. and Hooykaas, P.J.J. (1998) Cre/lox-mediated sitespecific integration of *Agrobacterium* T-DNA in *Arabidopsis thaliana* by transient expression of cre. *Plant Molecular Biology* **38**, 393–406.

Vergunst, A.C., Jansen, L.E.T. and Hooykaas, P.J.J. (1998) Site-specific integration of *Agrobacterium* T-DNA in *Arabidopsis thaliana* mediated by Cre recombinase. *Nucleic Acids Reearch* **26**, 2729–2734.

Verma, M.M. and Sindhu, P.S. (1995) Pigeonpea hybrids: historical development, present status and future perspective in Indian context. In: Rai, M. and Mauria, S. (eds.) *Hybrid Research and Development*. Indian Society of Seed Technology, Indian Agricultural Research Institute, New Delhi, pp. 121–137.

Waldron, C., Murphy, E.B., Roberts, J.L., Gustafson, G.D., Armour, S.L. and Malcom, S.K. (1985) Resistance to hygromycin B. A new marker for plant transformation studies. *Plant Molecular Biology* **5**, 103–108.

Wang, A.S., Evans, R.A., Altendorf, P.R., Hanten, J.A., Doyle, M.C. and Rosichan, J.L. (2000) A mannose selection system for production of fertile transgenic maize plants from protoplasts. *Plant Cell Reports* **19**, 654–660.

Wanjari, K.B. (1998) Utilization of alien species for development of cytoplasmic male sterility in pigeonpea. *Proceeding of Late Vasantrao Naik Memorial Seminar*. College of Agriculture, Nagpur, India.

Wanjari, K.B., Patil, A.N., Manapure, P., Manjayya, J.G. and Manish, P. (2001) Cytoplasmic male-sterility in pigeonpea with cytoplasm from *Cajanus volubilis*. *Annals of Plant Physiology* **13**, 170–174.

Weeks, T. (2000) Transformation of wheat with the *cyanamide hydratase* gene (09/518,988). Patent, U.S. Department of Agriculture, Agricultural Research Service. http://ott.arsusda.gov/Inv/a518988.html.

Wilmink, A. (1995) Activity of constitutive promoters in various species from the Liliaceae. *Plant Molecular Biology* **28**, 949–955.

Wolfenbarger, L.L. and Phifer, P.R. (2000) The ecological risks and benefits of genetically engineered plants. *Science* **290**, 2088–2093.

Xing, A.Q., Zhang, Z.Y., Sato, S., Staswick, P. and Clemente, T. (2000) The use of the two T-DNA binary system to derive marker-free transgenic soybeans. *In Vitro Cell Developmental Biology—Plant* **36**, 456–463.

Xuxiao, Z., Shiying, Y., Zhenghong, L., Jianping, G., Jinshui, X., Bingzhuang, Y., Wenlin, J., Fengjin, L., Chen, W. and Shumin, W. (2002) China-ICRISAT collaboration on pigeonpea research and development. *International Chickpea and Pigeonpea Newsletter* **8**, 35–37.

Yan, B., Reddy, M.S.S., Collins, G.B. and Dinkins, R.D. (2000) *Agrobacterium tumefaciens*-mediated transformation of soybean using immature zygotic cotyledon explants. *Plant Cell Reports* **19**, 1090–1097.

Yang, G., Rhodes, D. and Joly, R.J. (1996) Effects of high temperature on membrane stability and chlorophyll fluorescence in the glycinebetainedeficient and glycinebetaine containing maize lines. *Australian Journal of Plant Physiology* **23**, 437–443.

Yan, L. and Kerr, P.S. (2002) Genetically engineered crops: their potential use for improvement of human nutrition. *Nutrition Review* **60**, 135–141.

Yoder, J.I. and Goldsbrough, A.P. (1994) Transformation systems for generating marker-free transgenic plants. *Bio/Technology* **12**, 263–267.

Young, N.D., Mudge, J. and Ellis, T.N. (2003) Legume genomes: more than peas in a pod. *Current Opinion in Plant Biology* **6**, 199–204.

Zhang, H.X. and Blumwald, E. (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnology* **19**, 765–768.

Zhang, H.X., Hodson, J.N., Williams, J.P. and Blumwald, E. (2001) Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proceedings of the National Academy of Sciences of the USA* **98**, 6896–6901.

Zhao, Y., Aspinall, D. and Paleg, L.G. (1992) Protection of membrane integrity in *Medicago saliva* L. by glycinebetaine against the effects of freezing. *Journal of Plant Physiology* **140**, 541–543.

FURTHER READING

Saxena, K.B. (1999) *Pigeonpea in Sri Lanka* (in English, Summaries in English, French). ICRISAT, Patancheru.

Saxena, K.B. and Kumar, R.V. (2005) Improved pigeonpea hybrid parents for increased and stable production. Annual Report 2004, ICRISAT-Private Sector Pigeonpea Hybrid Parents Research Consortium, ICRISAT, Patancheru, AP, India, p. 35.

The Royal Society Policy and statement Report (2000) Transgenic plants and world agriculture. http://www.royalsoc.ac.uk/document.asp?tip=0&id=1447.