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Chickpea

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.), a self-pollinating diploid annual with $2n = 2x = 16$ chromosomes, is an important food legume crop throughout the world, especially in the developing countries. With over 10 million ha under cultivation in more than 30 countries in arid and semiarid areas of central, south, and southeast Asia, southern Europe, northern and eastern Africa, in the Americas and Australia, chickpea is second only to common bean (*Phaseolus vulgaris*) and third in production among the legumes. Chickpea is the only cultivated species belonging to the *Cicer* genus, which is a member of the Leguminosae family, Cicereae *Alef* tribe (van der Maesen, 1987). Commercially, the species is grouped into *desi* and *kabuli* types: *desi* chickpeas generally have small, colored seeds, whereas *kabulis* produce large, cream colored ones. *Kabulis* are usually utilized as whole grains while *desis* are decorticated and processed into flour. Chickpea is mainly used for human consumption and only a small proportion is used as feed. The chickpea seed is a good source of carbohydrates and proteins, which together constitute 80% of the total dry seed weight. The crude protein content of chickpea varies from 17–24% containing the essential amino acids like tryptophan, methionine, and cysteine. Chickpea is a cool season annual crop performing optimally in 70–80 °F daytime temperatures and 64–70 °F night temperatures. The crop produces good yields in drier conditions because of the deep tap root system. During 2002–2004, the global chickpea production was 8.0 million tons from an area of 10.1 million ha, giving an average productivity of 786 kg ha^{-1} (ICRISAT, 2007).

1.1 History, Origin, and Distribution

Chickpea with a moderately sized genome of around 750 Mbp (mega base pair) (Arumuganathan and Earle, 1991) evolved from its wild progenitor *Cicer reticulatum* through natural selection. Chickpea is one of the pulse crops domesticated in the Old World ca 7000 years ago (van der Maesen, 1987). Vavilov (1926) identified

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two primary centers of origin, Southwest Asia and the Mediterranean, and one secondary center of origin, Ethiopia. Based on cytogenetical and seed protein analysis, Ladizinsky and Adler (1976) considered *C. reticulatum* as the wild progenitor of chickpea and southern Turkey as the center of origin for the crop. Three wild annual *Cicer* species, *C. bijugum*, *C. echinospermum*, and *C. reticulatum*, closely related to chickpea, co-habit with the cultivar in this area. In general morphology, physiology, and genetics, *C. reticulatum* comes closest to the cultigen, making it a possible contender as the progenitor of chickpea. However, taking into account the polymorphic nature of ancestral populations and complex nature of domestication, one cannot rule out other possibilities, such as *C. reticulatum* and the cultigen sharing a common ancestor or a polyphyletic origin of chickpea. The *Cicer* species occur from sea level (e.g., *C. arietinum, C. montbretii*) to over 5000 m (*C. microphyllum*) near glaciers in the Himalayas. Chickpea is the only domesticated species under the genus *Cicer*, which was originally classified in the tribe Vicieae of the family Leguminosae and subfamily, Papilionoideae. Based on the pollen morphology and vascular anatomy, *Cicer* is now set aside from the members of Vicieae and is classified in its own monogeneric tribe, Cicereae *Alef*.

1.2 Classification and Crossability

The genus *Cicer* comprises 43 species and is divided into two subgenera. Chromosome number in *Cicer* species can be generalized as $2n = 2x = 16$. The cultivated species, *C. arietinum* is found only in cultivation and cannot colonize successfully without human intervention. The wild species (e.g., *C. reticulatum*, *C. bijugum*) occur in weedy habitats, mountain slopes among rubble (e.g., *C. pungens*, *C. yamashitae*), and on forest soils, in broad-leaf or pine forests (e.g., *C. montbretii, C. floribundum*) and can be grouped into annual and perennial forms. Studies on biosystematic relations between chickpea and its wild relatives following interspecific hybridization have been limited to the nine annual species, *C. arietinum*, *C. reticulatum, C. echinispermum, C. judaicum, C. pinnatifidum, C. bijugum, C. cuneatum, C. chorassanicum,* and *C. yamashitae*. Several groups have studied the genetic diversity and relatedness among annual *Cicer* species by employing hybridization, electrophoresis of seed storage proteins, isozymes, and molecular markers (Ladizinsky and Adler, 1976; van der Maesen, 1987; Kazan *et al.*, 1993; Labadi *et al.*, 1996; Sudupak *et al.*, 2002; Rajesh *et al.*, 2002; Nguyen *et al.*, 2004) leading to the classification of the wild species into three groups. The first group includes the primary and secondary crossability group, chickpea and its closest relatives (*C. reticulatum* and *C. echinospermum*); the second group (the annual tertiary group) consists of *C. pinnatifidum*, *C. judaicum*, and *C. bijugum*; while the third group includes mostly perennial tertiary species as well as two annual species *C. cuneatum* and *C. yamashitae*. Species within the primary gene pool (*C. arietinum*, *C. reticulatum*, and *C. echinospermum*) can be readily crossed usually generating fully fertile progeny; while species within the secondary gene pool (*C. bijugum*, *C. pinnatifidum*, and *C. judaicum*) can be successfully crossed with the cultigen *C. arietinum*, provided hybrid embryos are rescued. However, the progeny of crosses between primary and secondary gene pools are frequently sterile. Species within the tertiary gene pool (*C. cuneatum*, *C. yamashitae*, and others) have not yet been successfully crossed with the cultigen *C. arietinum*.

1.3 Consumers' Preference

During the past 20 years (1985–2004), the global chickpea area increased by 7%, yield by 24%, and production by 33%. Presently, the most important chickpea producing countries are India (64%), Turkey (8%), Pakistan (7%), Iran (3%), Mexico (3%), Myanmar (3%), Ethiopia (2%), Australia (2%), and Canada (1%) (ICRISAT, 2007). Although India produces a large variety of pulses, chickpea alone accounts for 43.2% of the total annual pulse production of 11.79 million tons. Chickpea has one of the highest nutritional compositions of any dry edible legumes and does not contain any specific major antinutritional factors. Chickpea seeds are eaten fresh as green vegetable, parched, fried, roasted, and boiled; as snack food, sweet, and condiments; seeds are ground and the flour can be used as soup, *dal*, and to make bread; prepared with pepper, salt, and lemon it is served as a side dish. *Dal* is the

split chickpea without its seed coat, dried and cooked into a thick soup or ground into flour for snacks and sweetmeats. Sprouted seeds are eaten as a vegetable or added to salads. Young plants and green pods are eaten like spinach. A small proportion of canned chickpea is also used in a few countries, and to produce fermented food. Animal feed is another use of chickpea in many developing countries. On an average, chickpea seed contains 23% protein, 64% total carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugar, and 3% ash (ICRISAT, 2007). Chickpea protein digestibility is the highest among the dry edible legumes. The lipid fraction is high in unsaturated fatty acids, primarily linoleic and oleic acids. Chickpea is also known for its use in herbal medicine and cosmetics. Chickpea meets 80% of its nitrogen requirement from symbiotic nitrogen fixation and can fix up to 140 kg N ha^{-1} from atmosphere. It leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health, long-term fertility and sustainability of the ecosystems.

1.4 Productivity Constraints

Greater and more stable yields are the major goals of plant breeding programs. Chickpea yields are usually an average of 400–600 kg ha^{-1}, but can potentially surpass 2000 kg ha^{-1}, and in experiments have attained 5200 kg ha^{-1}. Yields from irrigated crops are 20–28% higher than those from rainfed crops. Despite considerable international investment in conventional breeding, productivity of the crop has not yet been significantly improved. Currently, productivity of chickpea is low (world average being ~0.8 t ha^{-1}) (FAOSTAT, 2005) and has stagnated in recent years. Reasons underlying marginal improvements are series of biotic and abiotic stresses that reduce yield and yield stability. Especially *Ascochyta* blight and *Fusarium* wilt, pod borer, drought, and cold are the major constraints of yield improvement. The susceptibility of the plant to a foliar disease, *Ascochyta* blight, caused by the ascomycete *Ascochyta rabiei* and on the Indian subcontinent to the vascular disease *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* are the main constraints for increasing yield. Consequently, chickpea breeding aims at high yielding cultivars that combine long-lasting resistance against *Fusarium* wilt and *Ascochyta* blight with tolerance to abiotic stresses, such as drought and cold. Many other factors also prevent increase in chickpea yield such as, inadequate amount of fertilizers and pesticides, and some of the traditional methods of land preparation and lack of knowledge of elite seed material. Improving resistance to biotic and tolerance to abiotic stresses as well as a general increase in dry matter are major aims of chickpea breeders around the world. Among the abiotic factors, drought stands to be the number one problem in major chickpea growing regions because the crop is grown on residual moisture and the crop is eventually exposed to terminal drought. In West Asia and North African countries, low temperature causing freezing injury or death or delayed onset of podding reduces yield tremendously. Heat and salinity problems are relatively important following drought and cold stresses. In general, estimates of yield losses by individual pests, diseases, or weeds range from 5–10% in temperate regions and 50–100% in tropical regions.

1.5 Rationale for Transgenic Chickpea Breeding

During the past 30 years, the area under cultivation of chickpea has remained stagnant but the production has increased from 6.3 metric tons (during 1975) to 7.4 metric tons (during 2002) because of increase in its productivity from 614 kg ha^{-1} to 735 kg ha^{-1} during this period (ICRISAT, 2007). It is generally accepted that the average yield of chickpea is below its presumed potential, and efforts to improve the productivity of this crop by conventional breeding means have not been very effective. The major reason behind this is lack of sufficient and satisfactory levels of genetic variability within the cultivated chickpea germplasm. Many wild annual *Cicer* species, which possess a wealth of agronomically desirable genes, are sexually incompatible with the cultivated varieties. An effective and alternative approach is, therefore, to transfer genes from sources which are otherwise difficult to introduce through conventional breeding (Meeting Report, 2000). Major yield increases could be achieved

by development and use of cultivars that tolerate/resist abiotic and biotic stresses. In recent years, the wide use of early maturing cultivars that escape drought stress led to significant increase in chickpea productivity. In the Mediterranean region, yield could be increased by shifting the sowing date from spring to winter. However, this is hampered by the sensitivity of the crop to low temperatures and the fungal pathogen *A. rabiei*. Drought, pod borer (*Helicoverpa* spp.) and the fungus *F. oxysporum* additionally reduce harvests there and in other parts of the world. Tolerance to rising salinity will be a future advantage in many regions. Therefore, chickpea breeding focuses on increasing yield by pyramiding genes for resistance/tolerance to the fungi, pod borer, salinity, cold, and drought into the elite germplasm. As chickpea is a self-pollinating crop with a narrow genetic base, there is not much scope for heterosis breeding in this crop. Pure line breeding was recognized as a method of choice for developing new chickpea varieties. Due to limited genetic variability available in this crop, there is not much hope for overall yield increase in chickpea.

Marker-assisted selection (MAS) is another approach for crop improvement where genes of specific agronomic interest are tagged with DNA markers and are transferred to the host plant through conventional breeding. In chickpea, genomic maps have been constructed, genes for resistance to fungi such as *F. oxysporum* and *Ascochyta* blight have been tagged and attempts are being made for MAS as reviewed by Ford and Taylor (2006). However, knowledge of the inheritance of agronomic characters is a basic requirement to identify and integrate interesting genes in linkage maps and to utilize these maps for MAS of these characters to accelerate the development of new cultivars. Nevertheless, most genomic regions harboring genes for important traits are not yet sufficiently saturated with co-dominant markers to apply MAS in chickpea breeding programs. Development of transgenic chickpea is, therefore, identified as an important approach for its improvement.

2. DEVELOPING TRANSGENIC CHICKPEA

Though chickpea is an important grain legume, it suffers from heavy losses due to susceptibility to insect pests like pod borer, fungal pathogens, and low tolerance to drought and low temparature. As discussed earlier about the difficulty in developing new varities that are superior to the available ones due to limitations in conventional breeding, it is imperative to develop superiour varieties using transgenic technology. For develoment of transgenic plants, it is necessary to locate the genes those exibit particular traits from avilable germplasm, their isolation, making them suitable to transfer into the target plant (modifying them by adding marker gene, promoter sequence, and termination sequence) and its transformation followed by an efficient regeneration protocol.

2.1 Locating and Isolating the Genes of Interest

Since there are significant losses in yield due to attack from viruses, fungi, and insects in chickpea, several attempts are being made to transfer specific coat protein genes and insecticidal protein genes from viruses and bacteria to produce virus resistant and insect resistant chickpea. It is very important to identify a set of germplasm of donor gene pools selected from individual crops for gene transfer studies and crop improvement. The contrasting growing regimes of cultivated chickpea and its wild progenitor may have resulted in a different allelic repertoire at different loci in the wild and cultivated gene pools. In recent years, attempts have been made to use wild *C. reticulatum* as a genetic resource for chickpea improvement (Abbo *et al.*, 2002). The world collection of chickpea germplasm contains genotypes that remain uncultivated because of poor agronomic characteristics, but possess a high level of resistance against *A. rabiei* that is a severe and destructive fungal disease (Collard *et al.*, 2001). Agronomic characters of wild *Cicer* species are presented in Table 1.

Development of gene-based markers to resolve the problems related to limited utilization of wild *Cicer* species by chickpea breeding programs can be used to provide candidate gene markers for mapping of quantitative trait loci (QTLs) controlling important agronomic traits. An expressed sequence tag (EST) library was constructed using root tissue from two very closely related chickpea genotypes. A total of 106 EST-based

Table 1 Wild *Cicer* species with valuable agronomic characters[a]

Stress	Species with accessions exhibiting resistance or tolerance	Authors
Fusarium wilt	*C. reticulatum*[b], *C. echinospermum*[b], *C. pinnatifidum*[b], *C. juidacum*[b], *C. bijugum*[b]	Nene and Haware, 1980; Haware *et al.*, 1992; Kaiser *et al.*, 1994; Singh *et al.*, 1994; Infantino *et al.*, 1996; Singh *et al.*, 1998
Ascochyta blight	*C. reticulatum*[b], *C. echinospermum*[b], *C. pinnatifidum*[b], *C. juidacum*[b], *C. cuneatum*, *C. montbretti*, *C. anatolicum*	Singh *et al.*, 1981; Singh and Reddy, 1983; Haware *et al.*, 1992; Singh *et al.*, 1998; Stagmina *et al.*, 1998; Collard *et al.*, 2001
Botrytis gray mold	*C. bijugum*[b]	Haware *et al.*, 1992
Phytophthora	*C. echinospermum*[b]	Singh *et al.*, 1994
Cyst nematode	*C. reticulatum*, *C. pinnatifidum*[b], *C. bijugum*	Singh *et al.*, 1989; Singh and Reddy, 1991; Singh *et al.*, 1994, 1996, 1998
Leaf miner	*C. reticulatum*, *C. echinospermum*[b], *C. pinnatifidum*[b], *C. juidacum*[b], *C. bijugum*[b], *C. chorassanicum*[b], *C. cuneatum*[b]	Singh *et al.*, 1994, 1998
Bruchid (seed beetle)	*C. reticulatum*[b], *C. echinospermum*[b], *C. pinnatifidum*, *C. bijugum*[b], *C. Juidacum*[b], *C. cuneatum*	Singh *et al.*, 1994, 1998
Cold Drought	*C. reticulatum*, *C. echinospermum*, *C. bijugum*[b], *C. pinnatifidum*, *C. juidacum*, *C. microphyllum*	Chandel, 1984; Van Der Maesen and Pundir, 1984; Singh *et al.*, 1991, 1995, 1998; Kaiser *et al.*, 1993
Pea streak carlavirus	*C. microphyllum*, *C. anatolicum, C. canariense, C. microphyllum, C. oxyodon*	

[a]Reproduced from Croser *et al.*, 2003
[b]Higher rating species

markers were designed from 477 sequences with functional annotations and these were tested on *C. arietinum*. Forty-four EST markers were polymorphic when screened across nine *Cicer* species. Generated EST markers have detected high levels of polymorphism for both common and rare alleles. This suggests that they would be useful for allele mining of germplasm collections for identification of candidate accessions in the search for new sources of resistance to pests/diseases, and tolerance to abiotic stresses (Buhariwala *et al.*, 2005). Comparative biology and genomics are used to discover or validate the function of key genes. Resistance response to *Ascochyta* blight in four chickpea genotypes was studied using microarray technology and a set of chickpea unigenes, grasspea (*Lathyrus sativus* L.). ESTs and lentil (*Lens culinaris* Med.) resistance gene analogues. The four genotypes included resistant, moderately resistant, susceptible and wild relative of chickpea. The time course expression patterns of 756 microarray features resulted in the differential expression of 97 genes in at least one genotype at one time point (Coram and Pang, 2005, 2006).

Comparisons between genotypes resistant and susceptible to *A. rabiei* revealed potential gene "signatures" predictive of effective *A. rabiei* resistance. These genes included several pathogenesis-related proteins, SNAKIN2 antimicrobial peptide, proline-rich protein, disease resistance response protein DRRG49-C, environmental stress-inducible protein, leucine-zipper protein, polymorphic antigen membrane protein, Ca-binding protein, and several unknown proteins. The information generated enhances the understanding of this plant–pathogen relationship and may aid breeding programs directed toward the production of resistant cultivars (Coram and Pang, 2006). Bhattarai and Fettig (2005) reported that a wild relative of chickpea, *C. pinnatifidum*, is more tolerant than chickpea itself to various abiotic stresses, including drought. A complementary DNA (cDNA) clone encoding a dehydrin gene, *cpdhn1*, was isolated from a cDNA bank prepared from ripening seeds of *C. pinnatifidum*. The polypeptide deduced to correspond to this gene, *cpdhn1*, consists of 195 amino acid residues with a molecular mass of 20.4 kDa. Northern

blot analyses showed that *cpdhn1* expression was induced not only during seed development, but also in leaves in response to drought, chilling, and salinity and also to treatment with abscisic acid (ABA) or methyl jasmonate. The induction of *cpdhn1* expression by methyl jasmonate and ABA indicates that the gene may also be involved in the response to biotic stress. The CpDHN1 protein may thus improve the tolerance of chickpea to a variety of environmental stresses, both abiotic and biotic. In another work, Chen *et al.* (2004) identified three chickpea accessions, PI 559361, PI 559363, and W6 22589, showing a high level of resistance to *Didymella rabiei* (anamorph *Ascochyta*) pathotypes I and II, and can be utilized as resistance sources in chickpea breeding programs for resistance to *Ascochyta* blight. Previous studies on the genetics of chickpea resistance used undefined isolates of *D. rabiei*, and resulted in different genetic hypotheses involving one, two, or more resistance genes or QTLs (Hafiz and Ashraf, 1953; Singh and Reddy, 1983; Santra *et al.*, 2000; Tekeoglu *et al.*, 2000). Recent studies employing pathotype I or pathotype II isolates showed that resistance to pathotype I is conditioned by a single (major) gene, whereas resistance to pathotype II is conditioned by two or more independent loci (Udupa and Baum, 2003; Cho *et al.*, 2004). Another major factor limiting chickpea production is susceptibility to wilt disease (*F. oxysporum* f. sp. *ciceri*) that affects susceptible cultivars within 25 days after sowing and affected seedlings show drooping of leaves followed by complete collapse. Studies at ICRISAT, Patancheru, India, revealed that at least three genes are involved in conferring resistance to *Fusarium* wilt, and earliness or lateness of wilting depends directly upon the number of genes contributing to the trait (Upadhayaya *et al.*, 1983).

2.2 Designing the Transgene for Effective Expression

Transgenic plant technology has become a flexible platform for cultivar improvement as well as for studying gene function in plants. As the process of transformation protocols was elucidated important information came to light that made the development of efficient plant transformation vectors possible. It is known that the major determinant of gene expression (level, location, and timing) is the upstream of the coding region, which is termed as "the promoter", and is of vital importance. Any genes that are to be expressed in the transformed plant have to possess a promoter that will function in plants. This is an important consideration as many of the genes that are to be expressed in plants, particularly reporter genes and selectable marker genes are of bacterial origin. They, therefore, have to be supplied with a promoter that will drive their expression in plants. Transgenes also need to have suitable "terminator" sequences at their 3′ terminus to ensure that transcription ceases at the correct position. Failure to stop transcription can lead to the production of aberrant transcripts and can result in a range of deleterious effects, including inactivation of gene products, and increased gene silencing (Slater *et al.*, 2003b). Husnain *et al.* (1997) investigated the effects of different promoters, actin, cauliflower mosaic virus (CaMV), and Win6 on the expression of β-glucuronidase and kanamycin resistance marker genes introduced into zygotic embryos of chickpea. The CaMV promoter exhibited maximum efficiency at 44% followed by actin and Win6 promoters. Seed specific promoters are also useful for expression of foreign genes in the seeds. Shasany and Koundal (2000) isolated a *C. arietinum* legumin promoter, which shows similarity to chickpea 5′ part of the legumin structural gene and strong homology with pea promoter and pea *legumin A* gene sequence. This promoter can be utilized for expression of foreign genes in seeds of chickpea. A chimaeric, truncated bacterial *cryIA(c)* gene construct was developed for plant expression with the CaMV 35S promoter, *nos* terminator, an initiatory kozak sequence, and a translational enhancer sequence of tobacco mosaic virus. This *cryIA(c)* gene was co-transferred with a plasmid-containing *npt*II gene as the selection marker. The *cryIA(c)* gene was inhibitory to the development of the feeding larvae of *Heliothis armigera* Hubner, the chickpea pod-borer (Kar *et al.*, 1997).

The efficient production of transgenic plants requires stringent selection procedures supported by a selectable marker gene that confers resistance to agents, such as antibiotics or herbicides. Several such selection systems have recently been described for grain legumes, based on the marker genes neomycin phosphotransferase

II (*npt*II), hygromycin phosphotransferase (*hph*, *aph*IV or *hyg*), phosphinothricin acetyltransferase (*bar* or *pat*), conferring resistance to kanamycin, hygromycin, and the herbicide phosphinotricin (BASTATM), respectively. Transformation of chickpea was done by a seed specific alpha amylase inhibitor ($\alpha AI1$) gene from *P. vulgaris* and the *npt*II gene as selectable marker (Sarmah *et al.*, 2004). At present, only a small number or reporter genes are in wide use in plant transformation vectors, these being β-glucuronidase (*uidA* or *gus*), green fluorescent protein (*gfp*), luciferase genes (*lux* and *luc*) and, to a lesser degree, the chloramphenicol acetyltransferase gene (*cat*) (Slater *et al.*, 2003c). Senthil *et al.* (2004) described the stable integration and the expression of marker genes through three generations of transformed chickpea plants. pGIN1 binary plasmid construct included an intron-containing *uid*A gene (coding for GUS) (Vancanneyt *et al.*, 1990) under the control of a CaMV 35S promoter and a CaMV 35S terminator along with a *bar* gene (bialaphos resistance) driven by a CaMV 35S promoter element with a nopaline synthase (*nos*)pA terminator sequence. Earlier reports (Fontana *et al.*, 1993; Kar *et al.*, 1996) did not provide substantial evidence for the stable integration of transgenes into the progeny of the primary transformants. Krishnamurthy *et al.* (2000) obtained a single sterile T_1 plant containing the *GUS* gene, which had no detectable expression of the marker gene. Tewari-Singh *et al.* (2004) described T_0 transformants using three different selection systems, and one of these transformants gave transgenic progeny. Polowick *et al.* (2004) constructed a plasmid contained a bi-functional fusion gene conferring both *gus* and *npt*II activities, under the control of a 35S35SAMV promoter for chickpea transformation. The plasmid contains a bi-functional fusion gene (*gus*:*npt*II) conferring both *gus* and *npt*II activities (Datla *et al.*, 1991) with a 35S35SAMV promoter (Datla *et al.*, 1993), a NOS (nopaline synthase) terminator, and an intron (Vancanneyt *et al.*, 1990). Sarmah *et al.* (2004) reported transformation of chickpeas with a seed-specific chimaeric gene encoding bean $\alpha AI1$, using *npt*II as the selectable marker gene. The selectable markers *bar* and *npt*II have both been used for production of transformed chickpeas (Molvig *et al.*, 1995; Kar *et al.*, 1996). But Sarmah *et al.* (2004) used *npt*II that confers resistance to kanamycin, has proved to be a reliable selection system.

Despite the improvements made in vector design and advances in our understanding of both the mechanisms of transgene integration and plant gene expression, plant transformation is still in many ways an imprecise art. If more than one gene are in the vector, different promoters and terminators should be used for each of them. The use of the same promoter and/or terminator can lead to an increase in gene silencing. Multiple genes in one vector should not be immediately adjacent to each other, and should be in the same orientation. This avoids adjacent inverted repeats that cause plasmid instability in bacteria and increased gene silencing in plants (Slater *et al.*, 2003d).

2.3 *In Vitro* Regeneration: A Prerequisite for Genetic Transformation

A reproducible, reliable transformation system, combined with traditional breeding techniques, could aid in improving both the quality and yield of a given crop (Polowick *et al.*, 2004). One of the prerequisites for successful gene transfer in plants is the availability of a suitable protocol for transformation, which is compatible with *in vitro* plant regeneration methods of the targeted species (Kar *et al.*, 1996). Two methods of plant regeneration are widely used in transformation studies, i.e., somatic embryogenesis and organogenesis. In somatic embryogenesis, embryolike structures (embryoids), which can be developed into whole plants in a way analogous to zygotic embryo, are formed from somatic tissues. These somatic embryos can be produced either directly or indirectly. On the other hand, organogenesis relies on the production of organs, either directly from an explant or from a callus culture by suitable manipulation of growth medium. Key to success in integrating plant tissue culture technology into plant transformation strategies is the realization that a quick and efficient regeneration system must be developed. However, this system must also allow high transformation efficiencies from whichever transformation technique is adopted (Slater *et al.*, 2003a).

In vitro plant regeneration in chickpea has been achieved from shoot meristem through organogenesis (Bajaj and Dhanju, 1979; Sharma *et al.*, 1979), immature cotyledons (Shri and Davis,

1992) and through embryogenesis from immature cotyledons (Sagare *et al.*, 1993). *In vitro* plant regeneration in chickpea has also been reported from leaflet callus (Barna and Wakhlu, 1993; Kumar *et al.*, 1994). Regeneration studies using various explants were reported to produce shoots, either indirectly through a callus phase (Khan and Ghosh, 1984; Barna and Wakhlu, 1994; Altinkut *et al.*, 1997) or directly (Shri and Davis, 1992; Kar *et al.*, 1996; Subhadra *et al.*, 1998; Chakrabarty *et al.*, 2000; Shikha *et al.*, 2001; Sarmah *et al.*, 2004). High numbers of shoots per explant were produced from surface sterilized half embryonic axes attached to a cotyledon (Sarmah *et al.*, 2004). An efficient protocol for the regeneration of whole chickpea plants using embryonic axes after removal of the shoot and root tips as well as the axillary bud has been shown by Jayanand *et al.* (2003). The concentration of growth regulator was first indicated as critical for growth and morphogenesis by Skoog and Miller (1957). They reported that a higher cytokinin to auxin ratio promotes shoot growth in contrast to Bajaj and Dhanju (1979), who reported that only cytokinin has significant effect on the induction of multiple shoot. Rao (1990) and Fontana *et al.* (1993) reported higher regeneration frequency in chickpea in presence of 6-benzylaminopurine (BAP). Kar *et al.* (1996) achieved multiple shoot induction in chickpea using BAP and α-naphthaleneacetic acid (NAA) in regeneration medium. Thidiazuron in combination with BAP was also reported to produce multiple shoots in chickpea (Murthy *et al.*, 1996). Tissue culture and transformation conditions are strongly dependent on the genotype of explant (Adkins *et al.*, 1995). Genotype dependency of regeneration efficiency has been reported by Altinkut *et al.* (1997) using callus-derived plantlets and seedlings of chickpea. Reports on *in vitro* regeneration process of chickpea are presented in Table 2.

It is very important to identify a set of germplasm of donor gene pools selected from individual crops for gene transfer studies and crop improvement. The contrasting growing regimes of cultivated chickpea and its wild progenitor may have resulted in a different allelic repertoire at different loci in the wild and cultivated gene pools. In recent years, attempts have been made to use wild *C. reticulatum* as a genetic resource for chickpea improvement (Abbo *et al.*, 2002). The world collection of chickpea germplasm contains genotypes that remain uncultivated because of poor agronomic characteristics, but possess a strong capacity for resistance to *A. rabiei*. Wild relatives of *C. arietinum* also possess strong resistance, and may be bred with cultivated varieties to incorporate potential

Table 2 Reports on *in vitro* regeneration process of chickpea (*Cicer arietinum* L.) using various explants and different media compositions

S. no.	Explants	Optimized growth media	Processes of regeneration	References
1	Freeze preserved meristem	MS+11.4 mg l^{-1} IAA+2.3 mg l^{-1} Kin	Shoot meristem organogenesis	Bajaj and Dhanju, 1979
2	Immature cotyledon	B5+2,4-D+2,4,5-T+NAA+IAA +BA+Kin+Zeatin+ABA	Organogenesis from cotyledon like structure	Shri and Davis, 1992
3	Mature seeds	MS+10 mM TDZ	Direct organogenesis	Malik and Saxena, 1992
4	Immature cotyledon	MS+3 mg l^{-1} 2,4,5-T	Direct somatic embryogenesis	Sagare *et al.*, 1993
5	Leaf	MS+25 μM 2,4-D	Organogenesis from callus	Barna and Wakhlu, 1993, 1994
6	Meristem tips	DKWC+4.4 μM BA+0.05 μM IBA	Direct organogenesis	Bradt and Hess, 1993
7	Embryonic axes without apices	MS+B5 Vit+2.0 mg l^{-1} BAP+0.05 mg l^{-1} NAA	- do -	Kar *et al.*, 1996
8	Epicotyl	Basal medium+ BAP+Kin+IAA	Original paper was not found	Vani and Reddy, 1996
9	Cotyledonary nodes	MS+10 μM TDZ+ 10 μM L-Proline	Direct somatic embryogenesis	Murthy *et al.*, 1996
10	Hypocotyl	B5+BA	Direct organogenesis	Islam *et al.*, 1999
11	Internode	MS+B5 Vit+2,4-D+BAP+ NAA+Kin+IAA	Organogenesis from callus and direct organogenesis	Huda *et al.*, 2000
12	Embryo axes and germinating seeds	MS+2iP+TDZ+Kin+GA3+IBA +NAA	Direct organogenesis	Jayanand *et al.*, 2003

resistance genes (Collard *et al.*, 2001). Targeted transfer of genes from the wild *Cicer* species into the cultivated species would represent an elegant application of transformation technology. Chickpea transformation, now considered as a routine procedure in chickpea improvement, has brought the application of this technology much closer to reality (Fontana *et al.*, 1993; Hamblin *et al.*, 1998; Chakrabarty *et al.*, 2000; Krishnamurthy *et al.*, 2000; Sharma and Ortiz, 2000; Jaiwal *et al.*, 2001). The applicability of this technology will, however, depend on the identification of key genes, the number of genes controlling a particular character, and public acceptance of cultivars resulting from transformation technology (Croser *et al.*, 2003).

2.4 Genetic Transformation

Two methods, namely *Agrobacterium*-mediated and particle bombardment, have extensively been employed for genetic transformation of crop plants. Regeneration via the callus lends itself easily (compared to explants regenerating directly) to *Agrobacterium*-mediated transformations, while direct regeneration is more amenable for particle bombardment (Chandra and Pental, 2003). *Agrobacterium*-mediated transformation has been used successfully in grain legumes for over a decade (Christou, 1997). Reports on genetic transformation of chickpea are presented in Table 3. Early transformation experiments, which relied on callus cultures, failed due to poor shoot regeneration but demonstrated the potential of *Agrobacterium tumefaciens* as a transformation vector for chickpea (Islam *et al.*, 1994). Fontana *et al.* (1993) first reported successful chickpea transformation. They used embryonic axes as explants, which were co-cultivated with *A. tumefaciens* and produced at least two independent plants, which were confirmed at molecular level. Subsequently, using almost similar experimental protocols, the formation of multiple shoots from different genotypes and the production of primary transgenic plants were reported (Kar *et al.*, 1996; Krishnamurthy *et al.*, 2000).

In the above-mentioned transformation protocols, the transgenic plants were selected via multiple cycle *in vitro* on media containing kanamycin (Fontana *et al.*, 1993; Kar *et al.*, 1996) or phosphinothricin (Krishnamurthy *et al.*, 2000). Transformation frequencies and reproducibility in these early breakthroughs were low and limited their practical applicability. However, both transformation frequency and reproducibility have been improved recently in four separate studies (Polowick *et al.*, 2004; Sarmah *et al.*, 2004; Senthil *et al.*, 2004; Sanyal *et al.*, 2005), enabling the routine application of transformation technology to chickpea. In these protocols embryonic axes were used as explants. Cotyledonary explants containing half embryonic axes were used by Sarmah *et al.* (2004), while longitudinally sliced embryonic axes were used by Polowick *et al.* (2004) and Senthil *et al.* (2004). Sanyal *et al.* (2005) had precultured the explants (L2 layer) for 24 h on solidified MS-basal medium supplemented 1 mg l^{-1} BAP. The four systems appear equally useful and have some common elements like mature seeds are explant source, embryonic axes contain the target tissue, submersion of explants in *Agrobacterium* suspension followed by co-cultivation, frequent subcultures on selection medium, and transfer of rooted/grafted shoots to soil in the greenhouse. Although different groups have reported successful transformation of chickpea, the overall frequency of transformation is still very low (0.1–1.0%). Thus, to generate a sufficient number of transgenic lines with desired expression level a large number of explants need to be co-cultivated. In crops such as, maize and soybean, frequency of transformation has been dramatically enhanced with the use of Thiol compounds and L-cystine (Olhoft and Somers, 2001; Olhoft *et al.*, 2003). Similar efforts may also be made to improve the chickpea transformation efficiency. Use of super virulent strains of *A. tumefaciens* may be another option.

The biolistic gene (gene gun method) delivery where tungsten or gold particles are coated with the DNA that is to be used to transform the plant tissue has been successful in producing transgenic lines (Slater *et al.*, 2003e). There have been a few reports on production of transgenic chickpea plants using biolistic gene delivery. Kar *et al.* (1997) demonstrated the expression of *cryIA(c)* gene of *Bacillus thuringiensis* in transgenic chickpea plants. Explants and regeneration procedure were the same as their previous *Agrobacterium*-mediated transformation method, but only the gene construct and transformation

Table 3 Reports on genetic transformation of chickpea (*Cicer arietinum* L.) using various ex-plants, cultivars, methods of gene transfer, marker genes, and genes of interest

S. no.	Explant	Cultivar	Method of gene transfer	Genes transferred	Reference	Remark
1	Embryonic axis without apical meristem	Unknown	AT (LBA 4404)	*npt*II, *gus*	Fontana *et al.*, 1993	Low reproducibility, expression and integration of transgene in T_0 by Western and Southern analysis, respectively
2	- do -	ICCV-1, ICCV-6, Desi	AT (LBA 4404)	*npt*II, *gus*	Kar *et al.*, 1996	Low reproducibility, transgene integration by Southern analysis in T_0
3	Embryonic axis	ICCV-1, ICCV-6	MPB	*npt*II, *CryIAc*	Kar *et al.*, 1997	Low reproducibility, transgene transmitted to T_1 (PCR analysis)
4	- do -	PG 1, Chafa, Turkey, PG 12	AT (C58, C1, GV2260)	*npt*II, *pat*, *gus*	Krishnamurthy *et al.*, 2000	Low reproducibility, *gus* gene expressed in T_0, transmission into T_1 (PCR)
5	Cotyledons with half embryonic axis	Vijay	AT (AGL-1)	*npt*II, *Bt-Cry1Ac*	Das and Sarmah, 2003	Transgene transmitted to T_1 (PCR for *npt*II)
6	Embryonic axis	P 362, P 1042, P 1043	AT (EHA 101)	*npt*II, *gus, bar*	Tewari-Singh *et al.*, 2004	Low reproducibility, *gus* gene expressed in T_0, transmission to T_1 (PCR)
7	Cotyledons with half embryonic axis	Semsen	AT (AGL 1)	*npt*II, *αAI1*	Sarmah *et al.*, 2004	Reproducibility good, gene integration, transmission up to T_1 and expression in T_1 shown by Southern and Western analysis, respectively, high *αAI1* activity in T_1 seeds
8	Sliced embryonic axis	ICCV-5, H 208, ICCL 87322, K 850	AT (AGL 1)	*bar, gus, PGIP*	Senthil *et al.*, 2004	Reproducibility good, transmission and expression of *gus* up to T_3
9	- do -	CDC Yuma	AT (EHA 105)	*gus, npt*II	Polowick *et al.*, 2004	Reproducibility good, transmission and expression of *gus* up to T_3
10	L2 layer of cotyledonary nodes	C 235, BG 256, Pusa 362, Pusa 372	AT (LBA 4404)	*gus, npt*II, *CryIAc*	Sanyal *et al.*, 2005	Reproducibility good, transmission and expression of *npt*II and *Cry1Ac* in T_1 was shown, good *Cry1Ac* activity

method were different. Bio-Rad Biolistic 1000/He particle gun was used for delivering gene to the explants. Bacterial *cryIA(c)* gene was modified for plant expression. Transgenic kanamycin resistant chickpea plants were obtained through multiple shoot formation. Molecular analyses of the transformants indicated the presence of the transferred functional *cryIA(c)* gene in plant. The expression level of the *cryIA(c)* gene was inhibitory to the development of the feeding larvae of *Heliothis armigera* Hubner, the chickpea pod borer.

Transient expression of marker genes in the zygotic embryos of chickpea was demonstrated and conditions for optimum transient expression of *gus* and *npt*II genes were established. When 12 μgm of plasmid DNA per milligram of tungsten particles accelerated with helium discharge at a

60 kg cm^{-2} of mercury from a distance of 24 cm resulted in optimal transient expression of the *gus* and *npt*II gene in chickpea embryos (Husnain *et al.*, 1997).

2.5 Selection of Transformed Plants

The efficient production of transgenic plants requires stringent selection procedures supported by a selectable marker gene that confers resistance to agents such as antibiotics or herbicides. Several such selection systems have recently been described for grain legumes, based on the marker genes *npt*II, hygromycin phosphotransferase (*hph*, *aphIV*, or *hyg*), phosphinotricin acetyltransferase (*bar* or *pat*) conferring resistance to kanamycin, hygromycin, and the herbicide phosphinotricin (BASTATM), respectively (Chandra and Pental, 2003). In some cases, only a few transformed plants have been regenerated. Further optimizing the transformation parameters such as inoculation, co-culture condition, and selectable marker could increase transformation frequency. Although kanamycin has been the most favored selectable agent, it has not been proved an efficient selectable marker for grain legumes. The development of efficient uptake of selective agents by the regenerating tissues has increased recovery of transformed shoots, as has been shown by efficient selection in soybean on glufosinate-containing medium (Zhang *et al.*, 1999). The selectable markers *bar* and *npt*II have both been used for production of transformed chickpeas (Molvig *et al.*, 1995; Kar *et al.*, 1996). Sarmah *et al.* (2004) have used *npt*II in conjunction with kanamycin which has proved to be a reliable selection system where all of the plants selected based on kanamycin were proved to be transformed.

A rapid and reliable selection strategy has deterred chickpea improvement programs (Somers *et al.*, 2003). Tewari-Singh *et al.* (2004) developed an efficient and reliable nonantibiotic selection strategy using the *pat* and aspartate kinase (*AK*) genes for the production of transgenic chickpea. Kanamycin has been used for selection in most of the chickpea transformation studies reported (Fontana *et al.*, 1993; Kar *et al.*, 1996, 1997; Krishnamurthy *et al.*, 2000). There is only one report on the use of phosphinothricin (PPT) as a selective agent for chickpea (Krishnamurthy *et al.*, 2000), although it has been successfully used for selection in other legumes such as pea (Schroeder *et al.*, 1993, 1995; Bean *et al.*, 1997). Tewari-Singh *et al.* (2004) reported the use of the AK/LT selection system for the production of fertile transgenic chickpea. The results also showed that kanamycin and PPT can be used as selective agents for chickpea transformation. PPT was found to be a better selection marker than kanamycin as transgenic plants could be identified more easily and rapidly using the former.

3. FUTURE ROAD MAP FOR TRANSGENIC CHICKPEA

Although there has been an incremental increase in the productivity of chickpea during the past two decades using conventional breeding approaches, the productivity continues to be rather low, and far below the potential. The global chickpea demand in 2101 is estimated at 11.1 million metric tons, an increase of 29% from its level of 8.6 million metric tons during 2003–2004. Thus, a combination of productivity enhancement through genetic improvement might help achieving this target. Genes for transformation can be broadly divided into those that will be used to overcome the agronomic limitations (high yield potential, biotic and abiotic stresses) and the ones that could be used to enhance the value-added traits. Although major emphasis is currently being placed on improving the primary constraints, the manipulation of value-added traits, such as improving quality parameters like nutrition will be of much concern for chickpea improvement using transgenic technology. Transgenic technology could conceivably be used in chickpea for the introduction of disease–pest resistance; drought and salinity stress tolerance as well as value-added traits such as improved vitamins, micronutrients, and protein content thus enhancing the crop product value, quality, and safety. Current efforts include incorporating immunity or very high resistance to several viral and fungal diseases through transformation of chickpea cultivars that have very high demand for which no adapted resistant chickpea genotypes are available. Improved crop protection through the transfer and expression of disease resistance genes will decrease

or eliminate the usage of insecticides, pesticides, which are costly to the grower and may be harmful to the environment.

Finally, there are numerous traits that potentially could be manipulated with single or few gene introductions to produce more disease and pest resistant, drought and salinity tolerant, healthier, higher quality chickpeas.

3.1 Insect Resistance

The extent of crop losses by pod borer (*Helicoverpa armigera* Hubner) has been estimated to over US$ 1 billion annually where in chickpea it is estimated at over US$ 400 million in South Asia alone. Thus, improving chickpea resistance to this pest through genetic transformation is likely to contribute to sustainable crop protection and environmental conservation. For intractable pest problems such as *Helicoverpa*, the presumption is that no single tactic will suffice in itself to contain this pest. It has long been recognized that host plant resistance would be one of the most effective management options, but thus far, the levels of resistance in the available chickpea germplasm have been found to be very low. Genes encoding insecticidal proteins can be extensively used in generating chickpea transgenic plants for resistance to pod borer. Advancement in transgenic technology has made it possible to impart resistance to this devastating insect-pest of chickpea using different insecticidal genes from microorganisms, such as *Bt* crystal protein genes. Toxins genes from *Bt* deployed through transgenic plants are environmentally benign and incentive is to have improved resistance to this damaging pest while reducing reliance on synthetic pesticides. Transgenic chickpeas using *cry1Ab*, *cry1Ac*, and *SBTI* genes have already been developed at ICRISAT and are being subjected to insect bioassays and evaluated under contained field trials (K.K. Sharma, personal communication). Besides use of *Bt* genes, genes of plant origin like, lectins, diverse proteases, protease and amylase inhibitors also hold great promise in development of insect resistance in chickpea. Another possibility is to achieve an early and selective control of pod borer by targeting its specific physiology. Hence, insect chitinase-based strategy with different or multiple chitinase genes for the control of *H. armigera* is worth using for pod borer resistance in chickpea. These can also be used as companion transgenes, which, when engineered into the plant together with a *Bt* gene, multiply the effect of *Bt* δ-endotoxin by weakening the peritrophic membrane surrounding the insect midgut, thus improving resistance to the legume pod borer.

3.2 Disease Resistance

Chickpeas are susceptible to a number of fungal diseases, which affect the growth and productivity of this crop. Diseases of economic importance include *Fusarium* wilt (*F. oxysporum* f. sp. *ciceris*), *Ascochyta* blight (*A. rabiei*), botrytis gray mold (*Botrytis cinerea*), collar rot (*Sclerotium rolfsii*), and dry root rot (*Rhizoctonia bataticola*) among others. In chickpea, varieties need to be developed having multiple resistance against *Fusarium* wilt, collar and root rots, *Ascochyta* blight, and botrytis gray mold to succeed in farmers' fields. The way is now open to the testing of genetic transformation approaches designed to enhance fungal resistance in chickpeas. Hydrolytic enzymes such as chitinases and glucanases, which degrade the fungal cell wall components, also pose as attractive candidates for development of disease resistant chickpeas. The endochitinase derived from the biocontrol fungus *Trichoderma harzianum* appears to be particularly effective in inducing resistance to fungi and this version of endochitinase can also be used for genetic transformation purposes. Besides, another set of antifungal genes encoding for polygalacturonase-inhibiting proteins (PGIP) (that inhibit one or more key enzymes used by *Botrytis* and some other pathogenic fungi during invasion, thereby delaying the disease long enough for other defenses to take over) may be explored for transformation purposes in chickpea. Resistance to fungal diseases in chickpea can be achieved by using another group of plant derived secondary compounds known as phytoalexins, which have a direct inhibitory effect on the growth of fungal pathogens.

3.3 Biofortification

Improvement in nutritional quality traits is important for providing better nutrition to the consumers. In developed countries, there is already

a growing interest in use of chickpea as functional food or nutraceuticals and in developing dietary supplements (P.M. Gaur, personal communication). Chickpea is deficient in the sulfur-containing essential amino acids methionine and cystine, which lower its dietary and nutritional value. The nutritional quality of chickpea can be improved by either raising the level of sulfur-containing amino acids of storage proteins or by changing the proportion of methionine-rich proteins already present in the seeds. High methionine trait cannot be produced by conventional breeding methods because of its failure to detect genotypes containing desirable levels of methionine. There are reports on transferring a gene encoding a methionine-rich seed 2S albumin from sunflower to lupins. The albumin constituted 5% of the total protein in the seeds of the transgenic lupins and led to the doubling of the seed protein methionine. This approach might as well prove to be an alternative approach for developing methionine-rich chickpeas.

Although chickpea already contains higher amounts of carotenoids such as β-carotene, cryptoxanthin, lutein, and zeaxanthin than genetically engineered "golden rice" (Abbo *et al.*, 2005), the recent success in chickpea transformation technology might enable further enhancement according to the RDA (recommended dietary allowances) recommendations. The potential of such approaches is still largely unrealized but should yield seeds with enhanced nutritional quality for the future.

3.4 Enhancing Quality Traits

Chickpea does not contain any specific major antinutritional factors, such as ODAP in grasspea, vicin in faba bean, and trypsin inhibitors in soybean. However, A PA2-homologous protein, isolated from chickpea (*C. arietinum*), has been shown to have lectinlike properties and has been implicated in allergic responses in chickpea-sensitive individuals. This indicates that a reduction or removal of PA2 using genetic transformation approaches or gene silencing could lead to significant improvements in chickpea seed quality for food uses. Another negative factor ascribed to chickpea consumption is more flatulence due to higher concentrations of raffinose family oligosaccharides (RFOs) than any other dry legumes. Genetically manipulating the level of RFOs has been achieved by inhibiting the enzyme galactinol synthase, which catalyzes the first committed reaction in the pathway involving the synthesis of galactinol from UDP-Gal and myo-inositol. However, a better strategy involving the activation of α-galactosidase isolated from a thermophilic bacterium (*Thermotoga neapolitana*) for degradation of RFOs can be used considering the physiological importance of the RFOs during seed development and storage. Promoting the synthesis of a galactosyl cyclitol "ciceritol" in chickpea that is more slowly hydrolyzed by α-galactosidase than the RFOs could be another alternative for the reduction of RFOs thereby imparting protection during seed development and storage.

3.5 Abiotic Stress Resistance

Chickpea improvement for adaptation to abiotic stresses is crucial for stabilizing the yield in this major food legume crop. Genetic transformation provides prospects to enhance tolerance of chickpea to abiotic stresses including drought, salinity, and low temperature. Enhancing the production of chickpea under water deficits is vital as it is a rainfed crop. However, multigenic and quantitative nature of drought makes it difficult to breed for abiotic stress tolerance using conventional plant breeding. Knowledge of key genes involved in tolerance may allow genetic transformation of chickpea using such genes thus speeding up the breeding process. For example, efforts on enhancing the drought tolerance in chickpea are ongoing in ICRISAT, where transgenics carrying *P5CSF129A* gene encoding Δ^1-*pyrroline-5-carboxylate synthetase* driven by CaMV 35S promoter are being developed for overproduction of an osmolyte proline, which is known to have a role in osmotic adjustment and cell protection under water deficits. In another effort, *DREB1A* cDNA from *Arabidopsis thaliana,* capable of transactivating DRE-dependent transcription in plant cells under the control of stress inducible *rd29* promoter was introduced into a popular chickpea cultivar for improving drought and salinity tolerance in this important pulse crop (K.K. Sharma, unpublished results).

3.6 Nutrient Responsive Genotypes

With a shrinking area of quality arable land for agriculture and its increasing allocation to staple cereal or high value crops, it is unlikely that in future chickpea will be grown on lands that are better endowed with nutrients than on which it is grown at present. Mineral nutrient deficiency will, therefore, continue to be a major and increasing constraint to chickpea production. Mineral nutrient deficiencies commonly observed in major chickpea producing areas are: nitrogen (N) (due to suboptimal nitrogen fixation), phosphorus (P), sulfur (S), iron (Fe), zinc (Zn), and boron (B). It seems, therefore, realistic to expect some recovery of losses in yield due to nutrient deficiency through genetic improvement for increased nutrient acquisition. Detailed information about genetic aspects of plant mineral nutrition should be derived to augment research strategy for developing nutrient use efficient genotypes in chickpeas. Outputs of transgenic research in other crops, in due course of time, could be extended for enhancement of nutrient uptake mechanisms and salinity tolerance in chickpea.

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