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Chickpea: Crop Improvement under Changing Environment Conditions

B.K. Sarmah, S. Acharjee, and H.C. Sharma

Abstract

Chickpea, *Cicer arietinum*, is the second most important food legume in Asia after dry beans. Chickpea is an important source of protein, minerals, fiber, and vitamins in the diets of millions of people in Asia and Africa. Chickpea is also rich in essential amino acid lysine and deficient in sulfur-containing amino acids, methionine, and cysteine. Chickpea is mainly used for human consumption and only a small proportion is used as feed. It meets 80% of its N requirement from symbiotic nitrogen fixation and leaves substantial amount of residual nitrogen for the subsequent crops. It is a hardy crop well adapted to stress environments and a boon to the resource-poor marginal farmers in the tropics and subtropics. Average yields of chickpea are nearly 780 kg/ha, although farmers can harvest more than 2.5 tons/ha. The crop potential is nearly 5 tons/ha. Abiotic (drought, heat, and cold stress) and biotic (pod borers – *Helicoverpa armigera* and *Spodoptera exigua*, aphids – *Aphis craccivora*, leaf miner – *Liriomyza cicerina*, and bruchid – *Callosobruchus chinensis*) and diseases (*Fusarium* wilt, *Ascochyta* blight, *Botrytis* gray mold, and root rots) are the major stresses that constrain chickpea production in farmers fields. The major challenge is to reduce the losses due to biotic and abiotic constraints, and close the yield gap through crop improvement and crop management in future. A combination of productivity enhancement through varietal improvement, including biotechnological interventions, and integrated crop management is needed to realize the yield potential of this crop for improving food and nutritional security. Considerable progress has been made in developing high-yielding chickpea varieties to increase the productivity of this crop, while conventional breeding has been successfully used to breed disease-resistant varieties, little progress has been made in developing pod borer and drought-tolerant varieties, as the levels of resistance available in the cultivated germplasm are quite low. Wild relatives of chickpea have high levels of resistance to pod borer. Marker-assisted selection and genetic engineering of chickpea are being exploited to increase the levels of resistance/tolerance to these constraints and in future.

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15.1 Introduction

Global warming and climate change present a major challenge to the human beings as we heavily depend on natural resources, particularly agriculture, for food, feed, fodder, timber, fuel wood, and so on. Changes in climate will affect crop productivity and may degrade cultivable land [1, 2]. The natural calamities such as droughts, storms, floods, and heat waves might occur more frequently. A steady increase in temperature, decrease/increase in relative humidity, moisture stress, and increase in atmospheric carbon dioxide (CO_2) will also change the relative activity and abundance of insect pests, natural enemies, and their interaction with the host plants. Moreover, increased demand for biofuels will reduce the land available for cultivating food crops [3]. Therefore, there is need to enhance crop production by adopting modern tools of biotechnology and crop management to mitigate adverse effects of climate change.

Cereals, grain legumes, oilseeds, vegetables, and fruits are the major components of food for human beings. Among these, grain legumes play an important role in the dietary and nutritional needs of people, particularly in Asia, Africa, and Latin America. Among the many grain legumes consumed by people, chickpea is the most widely cultivated food legume in the world.

Chickpea (*Cicer arietinum* L.) is the second most important food legume in Asia after dry beans in terms of area, production, and consumption. Average annual chickpea area in the world is 10.7 million ha with a production of 8.2 million tons – Asia accounts for 90% of the area and 88% of the production of chickpea in the world. Chickpea is an important source of protein, minerals, fiber, and vitamins in the diets of millions of people in Asia and Africa. Chickpea has 23% protein, 64% total carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugar, and 3% ash [4]. Chickpea is rich in essential amino acid lysine and deficient in sulfur-containing amino acids, methionine, and cysteine. Chickpea also contains high amounts of carotenoids such as β-carotene, cryptoxanthin, lutein, and zeaxanthin [5]. Chickpea is mainly used for human consumption and only a small proportion is used as feed. The kabuli type (white or cream seed coat) is generally used as whole grains, while desi type (colored seed coat) is used as whole seeds, dehulled splits, or flour. Chickpea is used in preparation of a wide variety of dishes, popular snacks, soups sauces, enriched breads, and baby foods. Green chickpea leaves and twigs are eaten as a cooked vegetable and contain 4–8% protein [6]. Chickpea is also used as protein-rich animal feed and the vegetable biomass is highly valued as fodder in dry areas where grazing vegetation is scarce. Chickpea also plays an important role in improving soil fertility. It meets 80% of its N requirement from symbiotic nitrogen fixation and can fix up to 140 kg N/ha from air [7]. 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. Chickpea is a hardy crop well adapted to stress environments and a boon to the resource-poor marginal farmers in the tropics and subtropics.

Chickpea is an annual, self-pollinating, diploid ($2n = 2x = 16$) pulse crop with a genome size of 931 Mb [8]. It is the third most important grain legume, which is largely cultivated in Asia, Africa (East and North), and the Mediterranean Europe [9]. In recent years, it is also being cultivated in Australia, Canada, and the United States, largely for export to India. Annual chickpea production is 9.7 million tons (mt), followed by cowpea (5.7 mt), lentil (3.6 mt), and pigeonpea (3.5 mt). The major constraints in chickpea production are biotic stresses such as pod borers, *Helicoverpa armigera*, *Spodoptera exigua*, and *Helicoverpa punctigera* (in Australia), cutworm, *Agrotis* spp., aphid, *Aphis craccivora*, leaf miner, *Liriomyza cicerina*, *Fusarium* wilt, *Ascochyta* blight, Botrytis gray mold, and the abiotic stresses such as drought, heat, cold, and high salinity [10]. The estimated yield losses due to abiotic stresses (6.4 mt) are significantly higher than that due to biotic stresses (4.8 mt) [11]. Among the abiotic stresses of chickpea, drought causes a 40–50% reduction in yield globally [8]. The advanced biotechnological approaches suitable to mitigate the major biotic and abiotic stresses are in the following sections.

15.2
Abiotic Constraints to Chickpea Production

Abiotic stresses such as drought, salinity, and high temperature affect crop growth and productivity. The crop under adverse climatic condition shows morphological, physiological, biochemical, and molecular alterations. Drought is one of the major constraints to chickpea production throughout Asia, as the crop is largely grown under rainfed conditions during the post-rainy season or residual soil moisture, and experiences end-of-season drought (terminal drought). Areas prone to drought stress are expanding quite fast [12]. With the prediction of increasing water scarcity, terminal drought will continue to be the major constraint to chickpea production in several parts of the world. Often high-temperature stress during the reproductive phase occurs along with terminal drought stress, particularly in tropical short season-growing environments and during late sown conditions in most environments. Thus, it is important to combine tolerance to both drought and heat stress.

Legumes, in general, are sensitive to salt [13], and increasing use of irrigation has often converted the arable land into saline [14]. Approximately 22% of the agricultural land is saline [15]. Salinity is also a major limiting constraint to chickpea production in many parts of Asia. Saline soils contain sufficient neutral soluble salts (mainly sodium chloride and sodium sulfate) that adversely affect plant growth and grain yield.

Chilling temperatures during early reproductive growth have been reported to cause yield losses in chickpea in many parts of Asia. The plants continue to produce flowers, but fail to set pods when the mean daily temperature falls below 15 °C. Low temperatures also adversely affect size and viability of pollen and ovules, anther dehiscence, pollen germination and pollen tube growth, and fertilization [16]. In the Mediterranean region, the change from spring to winter sowing of chickpea has enhanced yields, but tolerance to low temperature during flowering requires further improvements [17].

A steady rise in the atmospheric concentrations of carbon dioxide has been observed, from 315 ppm in 1959 to 385 ppm at present [18]. The CO_2 concentration will continue to rise to as much as 500–1000 ppm by the year 2100 [19]. This will have a profound effect on crop growth and development [20] and alter the CO_2 metabolism in the plant. Under elevated atmospheric CO_2 concentrations, the nutritional quality of crops will be reduced due to less accumulation of proteins and nitrogen content in the grain and the leaves [21, 22]. Much of the protein in leaves is involved in assimilating carbon dioxide into sugars [23]. There is some yield advantage in chickpea under elevated CO_2, but a simultaneous reduction in nitrogen concentration may decrease the protein content and negate the reduction in protein [24].

15.3
Modern Crop Breeding Approaches for Abiotic Stress Tolerance

15.3.1
Drought, Salinity, and Low Temperature

Efforts are being made to exploit traits that are expected to play an important role in drought avoidance under receding soil moisture conditions by improving water availability to the plant through more efficient extraction of available soil moisture. Drought tolerance is a complex phenomenon involving many known and unknown pathways.

To improve drought tolerance, quantitative trait loci (QTLs) have been identified for stomatal conductance [25–27], transpiration efficiency [28, 29], osmotic adjustment [30–32], relative water content [30, 25], canopy temperature [31, 30], drought sensitivity index [25], leaf ABA [29], chlorophyll content [33], water use efficiency [34], root traits [35, 36] and some yield-related traits [28, 30, 31, 34, 37–40].

The most promising drought-tolerant line, ICC 4958, has 30% higher root biomass than the popular variety Annigeri [41]. Chandra *et al.* [42] identified molecular markers for a major QTL that accounts for 33% of the variation in root weight as well as in root length. New mapping populations have been developed and are being used to identify molecular markers for additional QTLs. Moreover, understanding physiological mechanisms that regulate drought tolerance, together with the associated regulatory genes, will facilitate crop improvement for water use efficiency and tolerance to drought. The possibility of investigating the response of genes to drought and other stresses by profiling of transcriptome, proteome, and metabolome will offer more information about this complex trait. Besides, studies on functional genomics of chickpea will also help in chickpea improvement.

Earlier studies have indicated limited variability in salinity tolerance in chickpea. However, a recent screening of over 250 germplasm accessions (including 211 accessions of minicore collection) and breeding lines/cultivars revealed wide variation in salinity tolerance [7]. Some accessions gave 10–20% higher yield under salinity stress than the most tolerant variety CSG 8962 released in India. These

results have renewed interest in breeding for enhanced salinity tolerance in chickpea.

Several breeding lines (e.g., ICCV 88502, ICCV 88503, ICCV 88506, ICCV 88510, and ICCV 88516) have been developed, which are able to set pods at lower temperatures (mean daily temperature between 12 °C and 15 °C). A pollen selection method has been developed and successfully applied for transferring cold tolerance from ICCV 88516 to the popular variety amethyst in Australia [43].

15.4 Genetic Engineering of Chickpea for Tolerance to Abiotic Stresses

15.4.1 Drought and Salinity

Abiotic stress is a complex trait that may involve many genes and, therefore, expression of more than one gene is essential to have reasonable tolerance to abiotic stresses. Development of genetically modified (GM) plants by the introduction and/or overexpression of selected gene(s) appear to be quite promising for chickpea improvement. For the development of abiotic stress-resistant transgenic plants, several stress-induced genes with known or unknown enzymatic or structural functions and regulatory proteins have been used. For genetic transformation, stress-induced genes with known functions such as water channel proteins, key enzymes for osmolyte biosynthesis (proline, betaine, sugars such as trehalose, and polyamines), detoxification enzymes, and transport proteins have also been used. Enzymes involved in the metabolic pathways appear more amenable to manipulations than the structural and developmental traits. Stress-induced regulatory proteins that are involved in stress response can be used to enhance tolerance to multiple stresses, including drought, salinity, and freezing [44].

A dehydration responsive element construct, where *DREBIA* gene from *Arabidopsis thaliana* is attached to a drought-responsive promoter (rd 29A) is being used to enhance drought tolerance in chickpea [45]. This construct is known to regulate a number of genes involved in the response to drought and other stresses, such as salinity and cold temperature. In the case of tobacco, wheat, and groundnut, overexpression of *DREB1A* has been shown to improve the drought as well as low-temperature stress tolerance [46–50].

The transgenic tobacco (*Nicotiana tabacum*) developed using *CAP2* gene from chickpea (*C. arietinum*) encoding a novel AP2 family transcription factor showed increase in leaf surface area and number of lateral roots. Transgenic plants were more tolerant to dehydration and salt stress than the wild-type plants, and expressed high steady-state transcript levels of abiotic stress–response genes *NtERD10B* and *NtERD10C* and auxin-response genes *IAA4.2* and *IAA2.5* [51].

Furthermore, introduction of an osmoregulatory gene *P5CSF129A* encoding the mutagenized Δ^1-pyrroline-5-carboxylate synthetase (*P5CS*) in chickpea showed accumulation of high proline (two–sixfolds). The transgenic events showed a

decline in transpiration at lower values of the fraction of transpirable soil water (dryer soil), and extracted more water than their untransformed parents. However, the overexpression of *P5CSF129A* gene caused less increase in transpiration efficiency, thereby indicating that the enhanced proline had little bearing on the components of yield architecture that are significant in overcoming the negative effects of drought stress in chickpea [52].

15.4.2 Elevated CO_2 Concentrations

Improving C_3 carbon fixation under temperature and drought conditions can be achieved by manipulation of Rubisco enzyme. The enzyme Rubisco dominates the limitation of C_3 fixation in conditions that restrict the supply of CO_2 such as high temperature or drought. The Rubisco enzyme is higher in plants comprised of chloroplasts encoding eight large subunits (LSU), while the nuclear DNA encoded eight small subunit (SSU) proteins. The large subunit (LSU) of Rubisco contains all the structural information necessary for catalysis, while the function of the small subunit (SSU) remains elusive. Genetic screening and site-directed mutagenesis have focused on the LSU of Rubisco as the catalytic site of the enzyme is on this subunit. Amino acid substitutions in several distinct areas of the Rubisco LSU that influenced the catalytic properties of Rubisco and genetic engineering have resulted in the production of an even less-efficient Rubisco [53–55]. Another alternative approach could be conversion of C_3 pathways to C_4 since C_4 pathway evolved in hot and arid regions in response to increasing O_2 levels as a mechanism to increase the CO_2 concentration at the site of Rubisco [56]. The C_4 system uses the enzyme phosphoenol pyruvate carboxylase (PEPC) to fix CO_2 from the atmosphere into C_4 in the mesophyll cells, which results in regeneration of phosphoenol pyruvate (PEP). Plants with C_4 pathway have a number of advantages, including high photosynthetic performance and high nitrogen and water-use efficiencies (WUE), allowing this group of plants to be highly productive in subtropical regions. Conventional plant breeding approaches have been used to try and transfer C_4 traits into C_3 plants, but these were unsuccessful. In the past decade, genes encoding enzymes in the C_4 pathway have been transferred to C_3 plants resulting in the production of the introduced enzyme [57–59]. However, to achieve this objective, it is necessary to use all the genes isolated from a C_4 species such as maize [60].

15.5 Biotic Constraints in Chickpea Production

15.5.1 Insect Pests

Nearly 60 insect species are known to feed on chickpea. The important insect pests damaging chickpea in different regions are cutworms (black cutworm –*Agrotis*

ipsilon (Hfn.) and turnip moth – *Agrotis segetum* Schiff, termites (*Microtermes obesi* (Holmgr.)), leaf feeding caterpillars (beet armyworm, *S. exigua* (Hub.) and hairy caterpillars *Spilarctia obliqua* Walker), leaf miners (*L. cicerina* (Rondani)), aphids (*A. craccivora* Koch), pod borers (cotton bollworm – *H. armigera* (Hub.), native budworm – *H. punctigera* (Wallengren)), and bruchids (Chinese bruchid – *Callosobruchus chinensis* L., bean bruchid – *Acanthoscelides obtectus* (Say.), pulse weevil – *Callosobruchus analis* F., and pulse bruchid – *Callosobruchus phaseoli* (Gylh.)) [61, 62]. The pod borer *H. armigera* and the aphid *A. craccivora* are the major pests of chickpea in the Indian Subcontinent. In the Mediterranean region, the most important pest is the leaf miner *L. cicerina*. *A. craccivora* is important as a vector of the chickpea stunt disease, while *C. chinensis* is the most dominant species in storage.

A continuous search is being made to identify resistant genotypes. More than 14000 chickpea germplasm accessions have been screened for resistance to *H. armigera* at ICRISAT, India, under field conditions. Several germplasm accessions (ICC 506EB, ICC 10667, ICC 10619, ICC 4935, ICC 10243, ICCV 95992, and ICC 10817) with resistance to *H. armigera* have been identified, and varieties such as ICCV 7, ICCV 10, and ICCL 86103 with moderate levels of resistance have been released for cultivation [63]. Progress has also been made in understanding the nature of gene action, and resistance to pod borer is largely controlled by additive gene action. Good combiners for pod borer resistance have also been identified [64]. However, most of these lines are highly susceptible to *Fusarium* wilt. Therefore, concerted efforts have been made to break the linkage by raising a large population of crosses between the lines with resistance to *H. armigera* and the lines resistant to wilt.

The extent of losses to chickpea in South Asia by this pest is estimated at over US$ 400 million [11]. In the storage condition, bruchids (*C. chinensis*) cause nearly 20–30% damage.

Global warming and climate change resulting in increased temperatures and reduced humidity will impact insect–host plant interactions in several complex ways. The effects of climate change on *H. armigera* have been investigated since the larvae of *H. armigera* have a wide host range [65]. Studies conducted on *H. armigera* under various ambient CO_2 concentrations (550–750 ppm) showed that larvae developed normally under elevated CO_2, and the adult moths lived longer, but laid fewer eggs [66]. However, when the larvae were reared on milky grains of spring wheat for three generations at ambient CO_2 concentration at 750 ppm, they exhibited slow growth in the second and third instars. It has been suggested that under elevated CO_2 concentrations, net damage by the cotton bollworm will be slow due to slow development [67]. Severity of the damage caused by *H. armigera* and the population relationship between *H. armigera* and its parasitoid wasp, *Microplitis mediator*, has also been also studied [68]. The results have suggested that there are no significant changes in wheat consumptions by the larvae or in the parasitism by the wasp under elevated CO_2 at 750 ppm.

Bt cotton being resistant to *H. armigera* was also evaluated under elevated CO_2 along with conventional cotton [69]. The results suggest that damage under elevated CO_2 might be higher, but there will be less pest population. Coll and

Hughes [70] reported that the *H. armigera* reared on pea plants (*Pisum sativum*) grown under elevated CO_2 at 700 ppm were significantly smaller than those reared on plants grown under ambient conditions. Furthermore, they also reported that the omnivorous bug, which feeds on plants but also preys on the bollworm, required prey to complete its development. The bugs performed best when the larvae reared under elevated CO_2, as the larvae were smaller and thus easily overcome by the predator. Elevated CO_2 may benefit generalist predators through increased prey vulnerability, which would put pest species under higher risk of predation. However, none of the above experiments were conducted under increased temperatures, which might level off the adverse effects of elevated CO_2 on *H. armigera* [71].

15.5.2 Diseases

The occurrence of pathogens is related to temperature, rainfall, humidity, radiation, and dew [72]. The movement of pathogens to their host plants depends on several factors, including its mode of dispersal and ability to survive on sources other than its primary host [73]. As dispersal of some pathogens is influenced by rain and winds [23], changes to these factors could also affect the spread of pathogens.

Fusarium wilt, Ascochyta blight, Botrytis gray mold, and root rot are the most important chickpea diseases [74, 75]. Fusarium wilt caused by *Fusarium oxysporum Schl. f. sp. ciceri* is the most important root disease of chickpea. The susceptible varieties can have up to 100% plant mortality. Ascochyta blight caused by *Ascochyta rabiei* is a highly devastating foliar disease of chickpea in northern India, Pakistan, and Central Asia. Botrytis gray mold caused by *Botrytis cinerea* is another important foliar disease of chickpea. It is a serious constraint to chickpea production in northern India, Nepal, Bangladesh, and Pakistan. Collar rot caused by *Sclerotium rolfsii* is becoming a serious problem in several parts of India, particularly central and southern India. Dry root rot caused by *Rhizoctonia bataticola* is a serious disease whenever the crop is exposed to moisture stress and temperature above 30 °C. Resistance to Fusarium wilt is necessary for all chickpea-growing areas, and all improved varieties have Fusarium wilt resistance. The foliar diseases, Ascochyta blight and Botrytis gray mold, continue to be a big threat to chickpea production in cooler and humid areas.

Muehlbauer and Kaiser [75] reported that the resistance to Ascochyta blight is multigenic. The pathogen evolves continuously, which makes it difficult to develop lines with stable resistance to this pathogen. The wild relatives of chickpea such as *Cicer ehinospermum, Cicer pinnatifidum, Cicer bijugum,* and *Cicer judacium* possess high levels of resistance to Ascochyta blight [76, 77].

The wilt caused by the soil borne fungi *F. oxysporum f. sp. ciceri* is an economically important disease of chickpea. Haware and Nene [77] identified seven distinct races of *Fusarium* in India. Of these races, race 1 is common in central India, and race 2 in northern India. However, race 3 and race 4 appear in various pockets of Punjab and Haryana. Race, 0, 5, and 6 were identified in Spain by Jimenez-Daiz *et al.* [78].

15.5.3
Biological Nitrogen Fixation

Biological nitrogen fixation offers an alternative means to increase plant-available nitrogen [7]. Nearly 20% of all N available to the crops is due to rhizobial N fixation [79]. Herridge *et al*. [80] estimated that 50% nitrogen fixed by a chickpea crop remains underground and is available to the following crop. However, symbiotic fixation of nitrogen is sensitive to even modest soil water deficits [81]. In the case of chickpea, the high nodulating selection ICC 4948 fixed more N and yielded 31% more than its low nodulating version [82]. Sufficient numbers of compatible rhizobia are often not naturally occurring in most of the soils where grain legumes are cultivated [83], and there is need for rhizobia application to seeds [84].

15.6
Modern Molecular Breeding Approaches for Biotic Stress Tolerance

15.6.1
Pod Borers

In the field, the pod borer *H. armigera* is a major threat to chickpea cultivation. Yoshida *et al*. [85] investigated the mechanisms of resistance to pod borers and found that oxalic acid and malic acid are the major components that govern resistance to *H. armigera*. Genotypes resistant to pod borer accumulated more oxalic acid on the leaves than the susceptible genotypes. Oxalic acid showed significant growth inhibition of the pod borer larvae when included in a semiartificial diet, while malic acid had no effect on larval growth.

Development of crop cultivars with resistance to pod borer is the most cost-effective and eco-friendly option for the control of *H. armigera*, particularly under subsistence farming conditions in the developing countries [86]. Availability of stable sources of resistance is a prerequisite to develop cultivars for resistance to insect pests. Screening of more than 14 000 germplasm accessions and breeding line has resulted in the identification of several genotypes with low to moderate levels of resistance to *H. armigera* for use in breeding programs. Some of these have also been found to be resistant in different agroclimatic zones under natural infestation. Germplasm accessions of wild relatives of chickpea (*C. bijugum*, *C. judaicum*, and *C. pinnatifidum*) have shown high levels of resistance to pod borer [87].

The chickpea cultivars and wild *Cicer* species have been found to differ significantly in their ability to inhibit *H. armigera* gut proteinases [88]. But none of the species offered complete protection against the pod borer by inhibiting the gut proteinases. The wild relatives of chickpea *C. bijugum* exhibited highest larval inhibition (36%), followed by *C. echinospermum* and *C. arietinum* (cv Vijay) (33%).

Stored chickpeas are highly susceptible to attack by the bruchids *Callosobruchus maculatus* and *C. chinensis*. Germplasm with some degree of resistance to bruchids

has been identified, but it appears to be correlated with undesirable physical characteristics of the seed coat. Bruchids-resistant chickpeas are usually consist of thick, dark color seed coat with altered chemical composition, but are less desirable for human consumption.

The preliminary linkage map based on interspecific crosses of *C. arietinum* × *Cicer reticulatum* and *C. arietinum* × *Cicer echinospermum* was made available by Gaur and Slinkard [89]. The mapping population derived from a cross between a wilt-resistant kabuli variety (ICCV 2) and a wilt-susceptible desi variety (JG 62) has been used to develop the first molecular map of chickpea based on an intraspecific cross [90]. A beginning has been made to identify molecular markers for resistance to *Helicoverpa* in chickpea. Mapping genes associated with resistance to *H. armigera* has been reported by Lawlor *et al.* [91]. High levels of resistance to *H. armigera* have been identified in wild relatives of chickpea (*C. bijugum, C. judaicum, and C. reticulatum*) [63], of which *C. reticulatum* can be easily crossed with the cultivated species to develop mapping populations to identify QTL associated with resistance to *H. armigera*.

A mapping population of 126 F_{13} RILs of ICCV 2 × JG 62 has been evaluated for resistance to *H. armigera*. The overall resistance score ($1 = <10$ leaf area and/or pods damaged, and $9 = >80\%$ leaf area and/or pods damaged) varied from 1.7 to 6.0 in the RIL population compared to 1.7 in the resistant check, ICC 506EB, and 5.0 in the susceptible check, ICCV 96029. The results indicated that there is considerable variation in this mapping population for susceptibility to *H. armigera*. Another RIL mapping population from the cross between Vijay (susceptible) × ICC 506EB (resistant) has also been evaluated for resistance to *H. armigera*. Interspecific mapping populations based on the crosses between ICC 3137 (*C. arietinum*) × IG 72933 (*C. reticulatum*) and ICC 3137 × IG 72953 (*C. reticulatum*) have also been developed, and putative QTLs linked to various components of resistance to *H. armigera* have been identified [92].

Based on interspecific genetic linkage map of chickpea (ICC 4958 × PI 489777) and phenotyping for resistance to *H. armigera* and *S. exigua* under field and greenhouse conditions, QTLs associated with resistance to pod borers have been identified [93] and can be used in conjunction with biochemical markers to develop cultivars with resistance to this pest. In addition, oxalic and malic acids and protease inhibitors have been identified as biochemical markers for resistance to *H. armigera* in cultivated chickpea and are being used to identify lines with resistance to this insect [94].

15.6.2
Ascochyta and Fusarium

Ascochyta blight appears to be controlled by several genes [95]. It has also been reported that there are two major complementary recessive genes for resistance to Ascochyta blight [96, 97]. Tekeoglu *et al.* [97] identified two major QTLs and one minor QTL from the interspecific crosses between *C. arietinum* and *C. reticulum*. Morjane *et al.* [98] performed genetic characterization of various isolates of *Ascochyta* of single field using DNA fingerprinting method and 12 haplotypes were

observed with varying frequencies. Santra *et al*. [99] developed a RAPD marker specific to Indian isolate. Coram and Pang [100] studied the molecular basis of the Ascochyta blight resistance in a highly resistant chickpea accession (ICC3996) and a susceptible cultivar (Lasseter) using microarrays. After inoculation with *A. rabiei*, a time-series expression patterns of 20 defense-related ESTs were studied and found upregulation or downregulation of 10 defense-related ESTs in ICC 3996 and/or Lasseter compared to the uninoculated control. Hierarchical clustering grouped the ESTs into different clusters. Three defense-related ESTs showed differential upregulation in ICC 3996 compared to Lasseter – a leucine zipper protein, SNAKIN2 antimicrobial peptide precursor, and elicitor-induced receptor protein.

Warkentin *et al*. [101] constructed a linkage map for resistance to Ascochyta blight and identified one QTL on each of LG3, LG4, and LG6, which accounted for 13%, 29%, and 12% of the total variation, respectively. Of these, three QTLs on LG4 and LG6 were in common with the previously reported QTL for Ascochyta blight resistance, whereas the QTL on LG3 was unique to this population.

The chickpea wilt caused by *F. oxysporum f. sp. ciceris* is one of the major factors limiting production of this pulse crop. The affected plants exhibit drooping crown, xylem, and stem discoloration and root rotting. Development of resistant varieties is thought to be the most viable strategy to overcome this problem. Muehlbauer and Kaiser [75] reported that resistance to different races of Fusarium is controlled by a single gene. Evaluation of both *desi*- and *kabuli*-type chickpea accessions revealed that almost 160 accessions were resistant to the fungus [102]. The wild accessions of *C. bijugum*, *C. judaicum*, *C. reticulatum*, and *C. ehinosperum* were resistant, while accessions belonging to *C. yamashitae* were susceptible. Breeding varieties resistant to Fusarium wilt were quite successful. However, some of the cultivars do not show resistance to all the races of the Fusarium wilt [103]. Mayer *et al*. [104] identified RAPD markers, UBC-170 and CS-27, located on same side of the locus, which were linked to resistance and susceptibility, respectively. Locus specificity of the primer UBC-170 was confirmed by allele-specific associated primer s (ASAPs). ISSR marker (UBC-855) linked to the gene conferring resistance to race 4 of Fusarium has been identified and appears to cosegregate with CS-27 [105]. Later, ISSR makers UBC 825 comprising dinucleotide repeats ($[AC]_8T$) was identified, which was 5.0 cM to the wilt resistant *Foc-4* gene [106].

15.6.3
Wide Hybridization

Wild relatives of chickpea are an important source of resistance to leaf miner *L. cicerina* and the bruchid *C. chinensis* [107]. Accessions belonging to *C. bijugum* (ICC 17206, IG 70002, IG 70003, IG 70006, 70012, IG 70016, and IG 70016), *C. judaicum* (IG 69980, IG 70032, and IG 70033), *C. pinnatifidum* (IG 69948), and *C. reticulatum* (IG 70020, IG 72940, IG 72948, IG 72949, and IG 72964) [108] have shown high levels of resistance to *H. armigera*. Some of the wild relatives of chickpea have different mechanisms than those in the cultivated types, which can be used in crop improvement to diversify the bases of resistance to this pest. High

levels of antibiosis were evident when *H. armigera* larvae were fed on leaves and pods, and the mechanisms are different from those in *C. arietinum* [109].

15.7 Application of Gene Technology

15.7.1 Pod Borers

Bacteria *Bacillus thuringiensis* consists of genes that encode several insecticidal proteins during sporulation (Cry or Cyt) and vegetative growth (Vips) proteins. Crickmore *et al*. [110] described more than 140 genes that produce Cry proteins, with specificities for Lepidoptera, Coleoptera, and Diptera. The Vips also possess toxic effects toward insects [111]. Vip3 is highly toxic to *Agrotis* and *Spodoptera* [105] and *H. armigera* [113].

Globally, insect-resistant crops have been one of the successful applications of plant genetic engineering technology. The first successful genetic transformation of chickpeas was reported in 1997 using the *cry1Ac* gene [114]. Later, transgenic chickpea expressing *Cry1Ac* [115–117] and *Cry2Aa* [118] genes were also generated. Recently, chickpea lines expressing pyramided *Bt* genes, *cry1Ac* and *cry1Ab* [117], have also been developed; however, the previous reports have suggested that *Cry1Ac* is more effective against *H. armigera*, and pyramiding two or more genes with different mode of action is preferred for effective pest management.

Another strategy, known as plant-delivered RNAi or in-plant RNAi, appears to be useful for the control of various insect pests, including the lepidopterans. A cytochrome P450 gene (*CYP6AE14*), which expresses in the midgut of *H armigera*, is proven to be a suitable candidate gene to control this pest. The *H. armigera* larvae fed on transgenic tobacco (*N. tabacum*) and *A. thaliana* plants expressing *dsRNA* of this gene have shown downregulation of cytochrome P450 gene and a significant larval growth retardation [119]. However, silencing of lepidopteran genes by RNAi has been found to be difficult. There may be several factors responsible for lower efficacy of RNAi in lepidopterans such as absence of RdRP orthologues in most insects [120], barriers to uptake of dsRNA, improper sorting of dsRNA during endosome trafficking to dsRNA-processing machinery, and so on [121]. Till date, the fate of the injected or ingested dsRNA in lepidopteran has not been understood. Recently, a tobacco rattle virus vector was found to be efficient in silencing a lepidopteran (*Manduca sexta*) gene *CYP6AE14* [121].

15.8 Conclusion

Chickpea cultivars with resistance to abiotic and biotic factors will form the backbone of chickpea production in future. The development and deployment of

chickpea plants with resistance to insects would offer the advantage of allowing some degree of selection for specificity effects, so that pests, but not the beneficial organisms, are targeted. Deployment of insect-resistant chickpeas will result in decreased use of chemical pesticides and increased activity of natural enemies and thus, higher yields. For pest management programs to be effective in future, there is a need for the following:

- Utilization of wild relatives of chickpea to diversify the genetic basis and thus, increase the levels of resistance to the target insect pests.
- Identification of quantitative trait loci associated with resistance to abiotic and biotic stress factors.
- Development of insect-resistant varieties through genetic transformation using genes with diverse modes of action.
- Combining resistance to insects with resistance to important diseases, drought, and cold tolerance.
- Focusing attention on crop management and insecticide resistance management.

References

1 Chrstensen, J.H. *et al.* (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon *et al.*), Cambridge University Press, New York, pp. 848–940.

2 Meehl, G.A. *et al.* (2007) in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon *et al.*), Cambridge University Press, New York, pp. 748–845.

3 Brown, M.E. and Funk, C.C. (2008) Food security under climate change. *Science*, **319**, 580–581.

4 William, P.C. and Singh, U. (1987) The chickpea: nutritional quality and the evaluation of quality in breeding programmes, in *The Chickpea* (eds M.C. Saxena and K.B. Singh), ICARDA, Aleppo, Syria, pp. 329–356.

5 Abbo, S., Molina, C., Jungmann, R., Grusak, M.A., Berkovitch, Z., Reifen, R., Kahl, G., Winter, P., and Reifen, R. (2005) Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet.*, **111**, 185–195.

6 Duke, J.A. (1981) *Handbook of Legumes of World Economic Importance*, Plenum Press, New York, p. 345.

7 Serraj, R. (2004) *Symbiotic Nitrogen Fixation: Prospectus for Enhanced Application in Tropical Agriculture*, Oxford, New Delhi.

8 Graham, P.H. and Vance, C.P. (2003) Legumes: importance and constraints to greater use. *Plant Physiol.*, **131**, 872–877.

9 Singh, K.B. *et al.* (1994) Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica*, **73**, 137–149.

10 Sharma, H.C. (2001) Crop protection compendium: *Helicoverpa armigera* (Hub.) in different agro-economical conditions. Chickpea in Nineties: Proceedings of the Second International Workshop on Chickpea, ICRISAT, pp. 181–189.

11 Ryan, J. (1997) A global perspective on pigeon pea and chickpea sustainable production systems: present status and future potential, in *Recent Advances in*

Pulses Research (eds A. Asthana and M. Ali), Indian Society for Pulses Research and Development, Kanpur, India, pp. 1–31.

12 Burke, E.J., Brown, S.J., Christidis, N. (2006) Modeling the recent evolution of global drought and projections for the twenty-first century with the Hadley centre climate model. *J Hydrometeor*, **7**, 1113–1125.

13 Munns, R., Husain, S., Rivelli, A., James, R., Condon, A., Lindsay, M., Lagudah, E., Schachtman, D., and Hare, R. (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. *Plant Soil*, **47**, 93–105.

14 Wang, W., Vinocur, B., and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, **218**, 1–14.

15 FAO (2004) World Agricultural Centre, FAOSTAT Agricultural Statistics DATA-Base Gateway.

16 Srinivasan, A., Saxena, N.P., and Johansen, C. (1999) Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): genetic variation in gamete development and function. *Field Crop Res.*, **60**, 209–222.

17 Millan, T., Clarke, H., Siddique, K., Buhariwalla, H., Gaur, P., Kumar, J., Gil, J., Kahl, G., and Winter, P. (2006) Chickpea molecular breeding: new tools and concepts. *Euphytica*, **147**, 81–103.

18 Keeling, R.F. Piper, S.C. *et al.* (2009) Atmospheric CO_2 records from sites in the SIO air sampling network, in *Trends: A Compendium of Data on Global Change*, Carbon Dioxide Information Analysis Center, Oak Ridge National, Laboratory, U.S. Department of Energy, Oak Ridge, TN.

19 IPCC (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, UK.

20 Ziska, L.H. (2008) Rising atmospheric carbon dioxide and plant biology: the overlooked paradigm, in *Controversies in Science and Technology, from Climate to Chromosomes* (eds D.L. KleinmanK.A. Cloud-Hansen *et al.*), Liebert, Inc., New Rochele, pp. 379–400.

21 Amthor, J.S. (2001) Effects of atmospheric CO_2 concentration on wheat yield: review of results from experiments using various approaches to control CO_2 concentration. *Field Crops Res.*, **73**, 1–34.

22 Pittock, B. (2003) *Climate Change: An Australian Guide to the Science and Potential Impacts*. The Australian Greenhouse Office.

23 Pal, M., Talwar, S., Deshmukh, P., Viswanathan, C., Khetarpal, S., and Kumar, P. (2008) Effect of elevated CO_2 on growth and photosynthetic characteristics of chickpea (*Cicer arietinum* L.). *Indian J. Plant Physiol.*, **13**, 4–8.

24 Kimball, B.A., Kobayashi, K., and Bindi, M. (2002) Responses of agricultural crops to free air CO_2 enrichment. *Adv. Agron.*, **77**, 293–368.

25 Sanguineti, M.C., Tuberosa, R., Landi, P., Salvi, S., Macaferri, M., Casarini, E., and Conti, S. (1999) QTL analysis of drought-related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration in field-grown maize. *J. Exp. Bot.*, **50**, 1289–1297.

26 Price, A.H., Young, E.M., and Tomos, A.D. (1997) Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). *New Phytol.*, **137**, 83–91.

27 Juenger, T.E., McKay, J., Hausmann, N., Keurenties, J., Sen, S., Stowe, K., Dawson, T., Simms, E., and Richards, J. (2005) Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana* II. QTL analysis of new mapping population, Kas-1 × Tsu-1. *Evolution*, **62**, 3014–3026.

28 Specht, J.E., Chase, K., Macrander, M., Graef, G.L., Chung, J., Markwell, J.P., Germann, M., Orf, J.H., and Lark, K.G. (2001) Soybean response to water: a QTL analysis of drought tolerance. *Crop Sci.*, **41**, 493–509.

29 Kholova, J., Hash, C.T., Lava Kumar, P., Yadav, R.S., Kocova, M. and Vadez, V. (2010). Terminal drought-tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br.] have high leaf ABA and limit transpiration at high vapour pressure deficit. *J. Exp. Bot.*, **61**1431–1440.

30 Diab, A.A., Teulat-Merah, B., This, D., Ozturk, N.Z., Benscher, D., and Sorrells, M.E. (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theor. Appl. Genet.*, **109**, 1417–1425.

31 Saranga, Y., Jiang, C-X, Wright, R.J., Yakir, D., and Paterson, A.H. (2004) Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. *Plant Cell Environ.*, **27**, 263–277.

32 Robin, S., Pathan, M.S., Courtois, B., Lafitte, R., Carandang, S., Lnceraas, S., Amante, M., Nguyen, H.T., and Li, Z. (2003) Mapping osmotic adjustment in an advanced back-cross inbred population of rice. *Theor. Appl. Genet.*, **107**, 1288–1296.

33 Shen, B., Zhang, J.Y., Zhang, K.Q., Dai, W.M., Lu, Y., Fu, L.Q., Ding, J.M., and Zheng, K.L. (2007) QTL mapping of chlorophyll contents in rice. *Agric. Sci. China*, **6**, 17–24.

34 Quarrie, S.A., Steed, A., Calestani, C., Semikhodskii, A., Lebreton, C., Chinoy, C., Steele, N., Pljevljakusic, D., Waterman, E., Weyen, J., Schondelmaier, J., Habash, D.Z., Farmer, P., Saker, L., Clarkson, D.T., Abugalieva, A., Yessimbekova, M., Turuspekov, Y., Abugalieva, S., Tubersosa, R., Sanguineti, M.C., Hollington, P.A., Aragues, R., Royo, A., and Doding, D. (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor. Appl. Genet.*, **110**865–880.

35 Chandra Babu, R., Nguyen, B.D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S.K., Palchamy, A., Sadasivam, S., Sarkarung, S., Wade, L.J., and Nguyen, H.T. (2003) Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. *Crop Sci.*, **43**, 1457–1469.

36 Li, Z., Mu, P., Li, C., Zhang, H., Li, Z., Gao, Y., and Wang, X. (2005) QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland japonica rice in three environments. *Theor. Appl. Genet.*, **110**, 1244–1252.

37 Moreau, L., Charcosset, A., and Gallais, A. (2004) Use of trial clustering to study QTL × environment effects for grain yield and related traits in maize. *Theor. Appl. Genet.*, **110**, 92–105.

38 Xu, J.L., Lafitte, H.R., Gao, Y.M., Fu, B.Y., Torres, R., and Li, Z.K. (2005) QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. *Theor. Appl. Genet.*, **111**, 1642–1650.

39 Xiao, Y.N., Li, X.H., George, M.L., Li, M.S., Zhang, S.H., and Zheng, Y.L. (2005) Quantitative trait locus analysis of drought tolerance and yield in maize in China. *Plant Mol. Biol. Rep.*, **23**, 155–165.

40 Dashti, H., Yazdi-Samadi, B., Ghannadha, M., Naghavi, M.R., and Quarri, S. (2007) QTL analysis for drought resistance in wheat using doubled haploid lines. *Int. J. Agric. Biol.*, **9**, 98–101.

41 Saxena, N.P., Krishnamurthy, L., and Johansen, C. (1993) Registration of a drought-resistant chickpea germplasm. *Crop Sci.*, **33**, 1424.

42 Chandra, S., Buhariwalla, H.K., Kashiwagi, J., Harikrishna, S., Rupa Sridevi, K., Krishnamurthy, L., Serraj, R., and Crouch, J.H. (2004) Identifying QTL-linked markers in marker-deficient crops. 4th International Crop Science Congress, September 26–October 1, 2004, Brisbane, Australia.

43 Clarke, H., Khan, T.N., and Siddique, K.H.M. (2004) Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. *Euphytica*, **139**, 65–74.

44 Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki,

K. (1999) Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.*, **17**, 287–291.

45 Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K. (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell*, **10**, 1391–1406.

46 Kasuga, M., Miura, S., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2004) A combination of the *Arabidopsis* DREB1A gene and stress inducible rd29A promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. *Plant Cell Physiol.*, **45**, 346–350.

47 Pellegrineschi, A., Reynolds, M., Pacheco, M., Brito, R.M., Almeraya, R., Yamaguchi-Shinozaki, K., and Hoisington, D. (2004) Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under greenhouse conditions. *Genome*, **47**, 493–500.

48 Behnam, B., Kikuchi, A., Celebi-Toprak, F., Yamanaka, S., Kasuga, M., Yamaguchi-Shinozaki, K., and Watanabe, K.N. (2006) The *Arabidopsis* DREB1A gene driven by the stress-inducible rd29A promoter increases salt-stress tolerance in proportion to its copy number in tetrasomic tetraploid potato (*Solanum tuberosum*). *Plant Biotechnol.*, **23**, 169–177.

49 Bhatnagar-Mathur, P., Devi, M.J., Serraj, R., Yamaguchi-Shinozaki, K., Vadez, V., and Sharma, K.K. (2004) Evaluation of transgenic groundnut lines under water limited conditions. *Int. Arch. Newsl.*, **24**, 33–34.

50 Bhatnagar-Mathur, P., Devi, M.J., Reddy, D.S., Vadez, V., Yamaguchi-Shinozaki, K., and Sharma, K.K. (2006) Overexpression of *Arabidopsis thaliana* DREB1A in transgenic peanut (*Arachis hypogaea* L.) for improving tolerance to drought stress (poster presentation). Arthur M. Sackler Colloquia on "From Functional Genomics of Model Organisms to Crop Plants for Global Health," April 3–5, 2006, National Academy of Sciences, Washington.

51 Shukla, D.C., Raha, R.K., Tripathi, V., and Chattopadhyay, D. (2006) Expression of Cap2 and APETALA2 family transcription factor from chickpea, enhances growth and tolerance to dehydration and salt stress in transgenic tobacco. *Plant Physiol.*, **142**, 113–123.

52 Bhatnagar-Mathur, P., Vadez, V., Jyostna Devi, M., Lavanya, M., Vani, G., and Sharma, K.K. (2009) Genetic engineering of chickpea (*Cicer arietinum* L.) with the *P5CSF129A* gene for osmoregulation with implications on drought tolerance. *Mol. Breed.*, **23**, 591–606.

53 Spreitzer, R.J. and Salvucci, M.E. (2002) Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. *Annu. Rev. Plant Biol.*, **53**, 449–475.

54 Andersson, I. and Taylor, T.C. (2003) Structural framework for catalysis and regulation in ribulose-1,5-bisphosphate carboxylase/oxygenase. *Arch. Biochem. Biophys.*, **414**, 130–140.

55 Parry, M.A.J., Andralojc, P.J., Mitchell, R. A.C., Madgwick, P.J., and Keys, A.J. (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *J. Exp. Bot.*, **54**, 1321–1333.

56 Sage, R.F. (2004) The evolution of C-4 photosynthesis. *New Phytol.*, **161**, 341–370.

57 Hausler, R.E., Hirsch, H.J., Kreuzaler, F., and Peterhansel, C. (2002) Overexpression of C-4-cycle enzymes in transgenic C-3 plants: a biotechnological approach to improve C-3-photosynthesis. *J. Exp. Bot.*, **53**, 591–607.

58 Leegood, R.C. (2002) C-4 photosynthesis: principles of CO_2 concentration and prospects for its introduction into C-3 plants. *J. Exp. Bot.*, **53**, 581–590.

59 Miyao, M. (2003) Molecular evolution and genetic engineering of C-4 photosynthetic enzymes. *J. Exp. Bot.*, **54**, 179–189.

60 Matsuoka, M., Furbank, R.T., Fukayama, H., and Miyao, M. (2001) Molecular engineering of C-4 photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **52**, 297–314.

61 Sharma, H.C., Srivastava, C.P., Durairaj, C., and Gowda, C.L.L. (2010) Pest management in grain legumes and climate change, in *Climate Change and Management of Cool Season Grain Legume Crops* (eds S.S. Yadav, D.L. McNeil, R. Redden, and S.A. Patil), Springer Science + Business Media, Dordrecht, The Netherlands, pp. 115–140.

62 Chen, W., Sharma, H.C., and Muehlbauser, F.J. (2011) *Crop Protection Compendium for Chickpea and Lentil Diseases and Pests*, The American Phytopathological Society, St Paul, MN, pp. 166.

63 Sharma, H.C. (ed.) (2005) *Heliothis/Helicoverpa Management: Emerging Trends and Strategies for Future Research*, Oxford, New Delhi, India, p. 469.

64 Sreelatha, E., Gowda, C.L.L., Gour, T.B., Sharma, H.C., Ramesh, S., and Upadhyaya, H.D. (2007) Genetic analysis of pod borer (*Helicoverpa armigera*) resistance and grain yield in *desi* and *kabuli* chickpeas (*Cicer arietinum*) under unprotected conditions. *Indian J. Genet. Plant Breed.*, **68**, 406–413.

65 CPC (2007) Crop protection compendium CAB.

66 Chen, F.J., Wu, G., Lu, J., and Ge, F. (2005) Effects of elevated CO_2 on the foraging behaviour of cotton bollworm, *Helicoverpa armigera*. *Insect Sci.*, **12**, 359–365.

67 Wu, G., Chen, F.J., and Ge, F. (2006) Responses of multiple generation of cotton bollworm *Helicoverpa armigera* Hubner, feeding on spring wheat to elevated CO_2. *J. Appl. Entomol.*, **130**, 2–9.

68 Yin, J., Sun, Y., Wu, G., Parajulee, M.N., and Ge, F. (2009) No effects on elated CO_2 on population relationship between cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), and its parasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae) *Agric. Ecosyst. Environ.*, **132**, 267–275.

69 Chen, F.J., Wu, G., Parajulee, M.N., and Ge, F. (2007) Long-term impacts of elevated carbon dioxide and transgenic Bt cotton on performance and feeding of three generations of cotton bolloworm. *Entomol. Exp. Appl.*, **124**, 27–35.

70 Coll, M. and Hughes, L. (2008) Effects of elevated CO_2 on an insect omnivore: a test for nutritional effects mediated by host plants and prey. *Agric. Ecosyst. Environ.*, **123**, 271–279.

71 Zvereva, E.L. and Kozlov, M.V. (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis. *Glob. Change Biol.*, **12**, 27–41.

72 Patterson, D.T., Westbrook, J.K., Joyce, R.J.V., and Rogasik, J. (1999) Weeds, insects and diseases. *Clim. Change*, **43**, 711–727.

73 Chakraborty, S., Murray, G., and White, N. (2002) *Impact of Climate Change on Important Plant Diseases in Australia*, Rural Industries Research and Development Corporation.

74 Nene, Y.L. and Reddy, M.V. (1987) Chickpea diseases and their control, in *The Chickpea* (eds M.C. Saxena and K.B. Singh), CAB International, Wallingford, pp. 99–125 (b)Nene, Y.L. and Reddy, M.V. (1987) Chickpea breeding, in *The Chickpea* (eds M. Saxena and K. Singh), CAB International, Wallingford, pp. 127–162.

75 Muehlbauer, F.J. and Kaiser, W.J. (1994) Using host resistance to manage biotic stresses in cool season food legumes. *Euphytica*, **73**, 1–10.

76 Singh, K.B. *et al.* (1998) Resistance to six races of *Ascochyta rabiei* in the world germplasm collection of chickpea. *Crop Sci.*, **33**, 186–189.

77 Haware, M.P. and Nene, Y.L. (1982) Races of *Fusarium oxysporum f. sp. ciceri*. *Plant Dis.*, **66**, 809–810.

78 Jimenez-Daiz, R.M., Trapero – Crass, A., Carbera De, L.A., and Coina, J. (1989) Races of *Fusarium oxysporum f.sp. ciceri* infecting chickpeas in southern Spain, in *Vascular Wilt Diseases of Plants* (eds E.C.

Tjamos and C.H. Beckman), NATO ASI Series H, Springer, pp. 515–520.
79 Smil, V. (1999) Nitrogen in crop production. *Global Biogeochem. Cycles*, **13**, 647–662.
80 Herridge, D.F., Peoples, M.B., and Boddey, R.M. (2008) Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil*, **311**, 1–18.
81 Sinclair, T.R., Purcell, L.C., King, C.A., Sneller, C.H., Chen, P., and Vadez, V. (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N_2 fixation. *Field Crops Res.*, **101**, 68–71.
82 Fried, M., Danso, S.K.A., and Zapata, F. (1983) The methodology of measurement of N_2 fixation by non-legumes as inferred from field experiments with legumes. *Can. J. Microbiol.*, **29**, 1053–1062.
83 Marufu, L., Karanja, N., and Ryder, M. (1995) Legume inoculants production and use in east and southern Africa. *Soil Biol. Biochem.*, **27**, 735–738.
84 Catroux, G. (1991) Inoculant quality standards and controls in France, in *Expert Consultation on Legume Inoculant Production and Quality Control* (ed. V.A. Thompson), FAO, Roma, Italy, pp. 113–120.
85 Yoshida, M., Cogill, S.E., and Wrightman, J.A. (1995) Mechanism of resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chick pea antibiotic factor. *J. Ecol. Entomol.*, **88**, 1783–1786.
86 Sharma, H.C., Gowda, C.L.L., Sharma, K.K., Gaur, P.M., Mallikarjuna, N., Bouhariwalla, H.K., and Crouch, J.H. (2003) Host plant resistance to pod borer, *Helicoverpa armigera* in chickpea, in Chickpea Research for the Millennium: Proceedings of the International Chickpea Conference, 20–22 January 2003 (eds R.N. Sharma, G.K. Shrivastava, A.L. Rathore, M.L. Sharma, and M.A. Khan), Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India, pp. 118–137.
87 Sharma, H.C., Singh, B.U., Hariprasad, K.V., and Bramel-Cox, P.J. (1999) Host-plant resistance to insects in integrated pest management for a safer environment. *Proc. Acad. Environ. Biol.*, **8**, 113–136.
88 Patankar, A.G., Harsulkar, A.M., Giri, A.P., Gupta, V.S., Sainani, M.N., Ranjekar, P.K., and Deshpande, V.V. (1999) Diversity in inhibitors of trypsin and *Helicoverpa armigera* gut proteinases in chickpea (*Cicer arietinum*) and its wild relatives. *Theor. Appl. Genet.*, **99**, 719–726.
89 Gaur, P.M. and Slinkard, A.E. (1990) Genetic control and linkage relations of additional isozyme markers in chickpea. *Theor Appl. Genet.*, **80**, 648–656.
90 Cho, S., Kumar, J., Shultz, J., Anupama, K., Tefera, F., and Muehlbauer, F.J. (2002) Mapping genes for double podding and other morphological traits in chickpea. *Euphytica*, **128**, 285–292.
91 Lawlor, H.J., Siddique, K.H.M., Sedgley, R.H., and Thurling, N. (1998) Improving cold tolerance and insect resistance in chickpea and the use of AFLPs for the identification of molecular markers for these traits. *Acta Hortic.*, **461**, 185–192.
92 Sharma, H.C., Clement, S.L., Muehlbauer, F., Kumar, S., Mahender, T., Varshney, R., Gaur, P.M., and Gowda, C. L.L. (2010) Potential for using morphological, biochemical, and molecular markers for resistance to insect pests in grain legumes. Vth International Congress on Legume Genetics and Genomics, July 2–8, Asilomar Conference Grounds, California, p. 150.
93 Clement, S.L., Sharma, H.C., Muehlbauer, F.J., Elberson, L.R., Mattinson, D.S., and Fellman, J.K. (2010) Resistance to beet armyworm in a chickpea recombinant inbred line population. *J. Appl. Entomol.*, **134**, 1–8.
94 Sharma, H.C., Rajeev, V., Gaur, P.M., and Gowda, C.L.L. (2008) Potential for using morphological, biochemical, and molecular markers for resistance to insect pests in grain legumes. *J. Food Legumes*, **21**, 211–217.

95 Vir, S. and Grewal, J.S. (1974) Physiologic specialization in *Ascochyta rabiei*, the causal organism of gram blight. *Indian Phytopathol.*, **27**, 355–360.

96 Santra, D.K., Tekeoglu, M., Ratnaparkhe, M., Kaiser, W.J., and Muehlbauer, F.J. (2000) Identification and mapping of QTLs conferring resistance to Ascochyta blight in chickpea. *Crop Sci.*, **40**, 1606–1612.

97 Tekeoglu, M., Santra, D.K., Kaiser, W.J., and Muehlbauer, F.J. (2000) Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. *Crop Sci.*, **40**, 1251–1256.

98 Morjane, H., Geistlinger, J., Harrabi, M., Weising, K., and Kahl, G. (1994) Oligonucleotide fingerprinting detects genetic diversity among *Ascochyta rabiei* isolates from a single chickpea field in Tunisia. *Curr. Genet.*, **26**, 191–197.

99 Santra, D.K., Singh, G., Kaiser, W.J., Gupta, V.S., Ranjekar, P.K., and Muehlbauer, F.J. (2001) Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of Ascochyta blight in chickpea. *Theor. Appl. Genet.*, **102**, 676–682.

100 Coram, T.E. and Pang, E.C.K. (2005) Isolation and analysis of candidate Ascochyta blight defence genes in chickpea. *Physiol. Mol. Plant Pathol.*, **66**, 201–210.

101 Warkentin, T.B., Tullu, T.D., and Vandenberg, A. (2007) Genetic mapping of Ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using a simple sequence repeat linkage map. *Genome*, **50**, 26–34.

102 Nene, Y.L. and Haware, M.P. (1980) Screening chickpea for resistance to wilt. *Plant Dis.*, **64**, 379–380.

103 Infantino, A., Porta-Puglia, A., and Singh, K.B. (1996) Screening wild *Cicer* species for resistance to Fusarium wilt. *Plant Dis.*, **80**, 69–101.

104 Mayer, M.S., Tullu, A., Simon, C.J., Kumar, J., Kaiser, W.J., Kraft, J.M., and Muelhlbauer, F.J. (1997) Development of a DNA maker for Fusarium wilt in chickpea. *Crop Sci.*, **37**, 1625–1629.

105 Ratnaparkhe, M.B., Tekeoglu, M., and Muehlbauer, F.J. (1998) Intersimple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. *Theor. Appl. Genet.*, **97**, 515–519.

106 Ratnaparkhe, M.B., Santra, D.K., Tullu, A., and Muehlbauer, F.J. (1998) Inheritance of inter-simple sequence-repeat polymorphisms and linkage with a Fusarium wilt resistance gene in chickpea. *Theor. Appl. Genet.*, **96**, 348–353.

107 Singh, K.B., Ocampo, B., and Robetson, L.D. (1998) Diversity for abiotic and biotic stress resistance in the wild annual *Cicer* species. *Genet. Resour. Crop Evol.*, **45**, 9–17.

108 Sharma, H.C., Pampapathy, G., Lanka, S.K., and Ridsdill-Smith, T.J. (2005) Potential for exploitation of wild relative of chickpea, *Cicer reticulatum* for imparting resistance to *Helicoverpa armigera*. *J. Econ. Entomol.*, **98**, 2246–2253.

109 Sharma, H.C., Pampapathy, G., Lanka, S. K., and Ridsdill-Smith, T.J. (2005) Antibiosis mechanism of resistance to legume pod borer, *Helicoverpa armigera* in wild relatives of chickpea. *Euphytica*, **142**, 107–117.

110 Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., and Dean, D.H. (1998) Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.*, **62**, 807–813.

111 Estruch, J.J., Carozzi, N.B., Desai, N., Duck, N.B., Warren, G.W., and Koziel, M. (1997) Transgenic plants: an emerging approach to pest control. *Nat. Biotechnol.*, **15**, 137–141.

112 Estruch, J.J., Warren, G.W., Mullins, M. A., Nye, G.J., Craig, J.A., and Koziel, M. G. (1996) Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci. USA*, **93**, 5389–5394.

113 Zhu, C., Ruan, L., Peng, D., Yu, Z., and Sun, M. (2006) Vegetative insecticidal protein enhancing the toxicity of *Bacillus thuringiensis* subsp kurstaki against *Spodoptera exigua* Lett. *Appl. Microbiol.*, **42**, 109–114.

114 Kar, S., Basu, D., Das, S., Ramkrishnan, N.A., Mukherjee, P.,

Nayak, P., and Sen, S.K. (1997) Expression of cry1Ac gene of *Bacillus thuringiensis* in transgenic chickpea plants inhibits development of borer (*Heliothis armigera*) larvae. *Transgenic Res.*, **15**, 473–497.

115 Sanyal, I., Singh, A.K., Kaushik, M., and Amla, D.V. (2005) *Agrobacterium*-mediated transformation of chickpea (*Cicer arietinum* L.) with *Bacillus thuringiensiscry1Ac* gene for resistance against pod borer insect *Helicoverpa armigera*. *Plant Sci.*, **168**, 1135–1146.

116 Indurker, S., Misra, H.S., and Eapen, S. (2007) Genetic transformation of chickpea (*Cicer arietinum* L.) with insecticidal crystal protein using particle gun bombardment. *Plant Cell Rep.*, **26**, 755–763.

117 Mehrotra, M., Singh, A.K., Sanyal, I., Altosaar, I., and Amla, D.V. (2011) Pyramiding of modified Cry1Ab and Cry1Ac genes of *Bacillus thuringiensis* in transgenic chickpea (*Cicer arietinum* L.) for improved resistance to pod borer insect *Helicoverpa armigera*. *Euphytica*, **182**, 87–102.

118 Acharjee, S., Sarmah, B.K., Kumar, P.A., Olsen, K., Mahon, R., Moar, W.J., Moore, A., and Higgins, T.J.V. (2010) Expression of a sequence-modified *cry2Aa* gene for resistance to *Helicoverpa armigera* in chickpea (*Cicer arietinum* L.). *Plant Sci.*, **178**, 333–339.

119 Mao, Y.B., Cai, W.J., Wang, J.W., Hong, G.J., Tao, X.Y., Wang, L.J., Huang, Y.P., and Chen, X.Y. (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat. Biotechnol.*, **25**, 1307–1313.

120 Gordon, K.H.J. and Waterhouse, P.M. (2007) RNAi for insect-proof plants. *Nat. Biotechnol.*, **25**, 1231–1232.

121 Kumar, P., Pandit, S.S., and Baldwin, I.T. (2012) Tobacco rattle virus vector: a rapid and transient means of silencing *Manduca sexta* genes by plant mediated RNA interference. *Plos One*, **7**, e 31347.