

Genetic Enhancement of Stress Tolerance in Chickpea: Present Status and Future Prospects

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Though there has been incremental improvement in the average yield of chickpea during the past two decades, it still remains very low ($\sim 0.8 \text{ t ha}^{-1}$) and is unstable. Several biotic and abiotic stresses constrain productivity. Drought, cold and salinity are the most important abiotic stresses, whereas *Fusarium* wilt, *Ascochyta* blight, *Botrytis* gray mold and *Helicoverpa* pod borer are important biotic constraints. Thus, breeding efforts in chickpea have largely focused on development of varieties resistant/tolerant to these stresses. To date developing short duration varieties has been the most effective strategy for minimizing losses from terminal drought stress although drought avoidance through large and deep roots is a promising avenue to stabilize yield under terminal drought. Large genetic variation has been observed in chickpea germplasm for root traits, such as rooting depth, density and biomass that can help the plant avoid drought stress by increasing its effective water capture. Efforts are being made at ICRISAT to incorporate these traits to enhance drought tolerance. The pollen selection method appears promising in identifying chilling tolerant genotypes and in breeding for chilling tolerance. The recent screening of chickpea mini-core collection and breeding lines/cultivars at ICRISAT has revealed large variation for salinity tolerance. Excellent progress has been made in the development of varieties with durable and high levels of resistance to *Fusarium* wilt. There has also been good progress in enhancing resistance to *Ascochyta* blight by pyramiding resistance genes from diverse germplasm sources. But the other stresses still remain a challenge to chickpea scientists due to low levels of resistance in the germplasm. Some wild *Cicer* species have been identified to have high antibiosis for *Helicoverpa*. Efforts are being made at ICRISAT to enhance *Helicoverpa* resistance by combining different mechanisms of resistance available in the cultivated and the wild species.

Molecular markers for stress tolerance genes are being identified so that these can be used for marker-assisted selection (MAS). Markers are now available for some of the major genes controlling resistance to *Fusarium* wilt and *Ascochyta* blight and a major QTL for

root traits. Applications of transgenic technologies are being explored for enhancing the levels of tolerance/resistance to the stresses for which sources with high levels of resistance are not available in the cultivated germplasm and cross compatible wild relatives. Transgenics with genes coding Bt toxin and soybean trypsin inhibitor (*SbTI*) have already been developed and are currently being evaluated under controlled conditions for resistance to pod borer. Other chickpea transgenics are at an earlier stage of development and include those carrying antifungal genes for enhancing resistance to fungal diseases, and drought responsive elements (*DREB1A*) and *P5CSF* gene for enhancing drought, salinity and cold tolerance. These recent advances in the development of transgenic products and molecular marker tools for stress tolerance genes are expected to accelerate the progress in improving tolerance of chickpea to stresses, which is very much needed for enhancing and stabilizing chickpea production.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important food legume globally and an important source of dietary protein for millions of people in the developing countries. During 2004, chickpea was grown in 46 countries on an area of 10.4 million ha, and 97.4% of this area was in the developing countries (FAO Stat 2004, <http://faostat.fao.org/faostat/>). The 2.6% chickpea area contributed by the developed countries was largely targeted for chickpea export to developing countries. Thus, most of the world's chickpea is consumed in the developing countries.

During the past 20 years (1985-2004), the global chickpea area increased by 7% (from 9.7 to 10.4 m ha), yield increased by 24% (663 to 826 kg ha⁻¹), and production increased by 33% (6.6 to 8.6 m t) (FAO Stat 2004, <http://faostat.fao.org/faostat/>). The productivity growth rate of 1.6% per year recorded for chickpea is very low. Moreover, there were wide fluctuations in yield from year to year.

There are several constraints to chickpea productivity. The crop is largely grown rainfed in marginal environments with inadequate inputs and there are several abiotic and biotic stresses that constrain productivity. Extensive efforts have been made in identifying sources of resistance/tolerance to these stresses and breeding for stress tolerance. This paper highlights recent developments and future prospects of chickpea improvement for major abiotic and biotic stresses.

ABIOTIC STRESSES

Drought

Chickpea is generally grown in the *rabi* (post-rainy) season on residual soil moisture and often experiences drought during the end of the season (terminal drought). Development of early maturing varieties has so far been the most effective strategy for drought escape. Availability of large number of short-duration high yielding varieties has led to increase in area and productivity of chickpea in central and southern India (latitudes 12° N to 24° N). This is particularly important for *kabuli* chickpeas, which were earlier confined to cooler areas with longer crop growing season. Several short-duration *kabuli* varieties have been

released for central and southern India (e.g. ICCV 2, KAK 2, JGK 1, Phule G 95311), which have significantly expanded *kabuli* chickpea area in these regions. The super-early lines (e.g. ICCV 96029, ICCV 96030) developed at ICRISAT have further enhanced opportunity for chickpea cultivation in new niches, such as catch crop between rice and wheat cropping sequence (Sandhu *et al.*, 2002).

Drought avoidance is considered as another major avenue to improve the tolerance of chickpea to drought. Indeed, root traits, particularly rooting depth and root biomass, are expected to play an important role in drought avoidance in receding soil moisture conditions by improving water availability to the plant through more efficient extraction of available soil moisture. ICC 4958, the most promising drought tolerant line identified at ICRISAT, was found to have 30% higher root biomass than the popular variety Annigeri (Saxena *et al.*, 1993). Recent studies conducted at ICRISAT on root growth parameters of 12 contrasting chickpea genotypes during two seasons (2000/01, 2001/02) showed a clear positive relationship between root biomass (at 35 DAS) and the seed yield in both the seasons (Serraj *et al.*, 2004a; Kashiwagi *et al.*, 2005). The broad sense heritability for root biomass was high (67.4%), suggesting that selection for this trait should be effective. However, the laborious methods involved in digging and measuring roots make it difficult to select for root traits in a segregating population.

Molecular markers closely linked with major quantitative trait loci (QTLs) controlling root traits can facilitate marker-assisted selection (MAS) for root traits. ICRISAT has developed 257 recombinant inbred lines (RILs) from Annigeri \times ICC 4958 cross and characterized these for root traits and SSR markers. An SSR marker (TAA 170) was identified for a major QTL that accounted for 33.1% of the variation for root weight and 33.4% of the variation for root length (Chandra *et al.*, 2004). Recently, the chickpea mini-core collection was evaluated for root traits and wide variation was observed for root depth and biomass. (Krishnamurthy *et al.*, 2003, Serraj *et al.*, 2004a). Accessions showing larger variation than that found between Annigeri and ICC 4958 were selected for development of new mapping populations. These included ICC 8261 and ICC 4958 with high root depth and biomass and ICC 283 and CC 1882 with low root depth and biomass. Two crosses (ICCV 283 \times ICC 8261, ICCV 4958 \times ICC 1882) were made and about 260 RILs were developed from each cross. These mapping populations are expected to facilitate identification of markers for additional QTLs for root traits.

ICRISAT has developed an EST library using a subtractive suppression hybridization (SSH) approach for isolation and characterization of root-specific genes differentially expressed between ICC 4958 and Annigeri. A total of 2,858 EST sequences were analyzed and the information on these is available at <http://www.icrisat.org/gt1/cpest/home/asp> (Jayashree *et al.*, 2005). This is a valuable resource for candidate gene mapping, allele mining of chickpea germplasm and development of single nucleotide polymorphism (SNP) markers from the most promising EST markers (Millan *et al.*, 2006).

Another approach being used at ICRISAT for enhancing drought tolerance in chickpea is the development of transgenics, using either promoters regulating a cascade of genes or single genes involved in stress response. Transgenics have already been developed with a dehydration responsive element construct, where *DREB1A* gene is attached to a drought-responsive promoter (rd 29A). 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. The T_1 plants are undergoing molecular characterization. Another gene *P5CSF-129A* has been used that increases proline accumulation in the plant and improves its tolerance to osmotic stress. Some selected transgenic plants have shown up to 5-fold increase in proline content. T_3 generation of these plants will soon be evaluated for various physiological parameters.

Low temperature

The freezing temperatures (mean daily temperature $<1.5^{\circ}\text{C}$) and chilling temperatures (mean daily temperature between -1.5°C to 15°C) have been demonstrated to be important constraints to chickpea production in certain geographical regions. The winter-sown chickpea in West Asia and North Africa (WANA) often experiences freezing temperatures during seedling and early vegetative stages of crop growth and chilling temperatures at the early reproductive growth stage. Freezing temperature reduces vigor and vegetative biomass, whereas the chilling temperature at flowering leads to flower and pod abortion. Thus, the cultivars for winter sowing in WANA need to have cold tolerance both at seedling and flowering stages. Screening of a large number of germplasm accession by ICARDA has led to identification of several cold tolerant lines (Singh *et al.*, 1981, 1984, 1995). The best sources of cold tolerance in the cultivated species were ILC 8262, ILC 8617 and FLIP 87-82C with a consistent score of 3 (on 1 to 9 scale, going from high to low tolerance) over years and locations (Singh *et al.*, 1995). Twenty-one accessions of *C. bijugum* and two accessions of *C. reticulatum* were found to have higher level of cold tolerance (score 2) than the cultigen (Robertson *et al.*, 1995). Of these species, *C. reticulatum* can be crossed with the cultigen and should be exploited in breeding for cold tolerance.

Chilling temperatures during early reproductive growth have been reported to cause yield losses in chickpea also in parts of the Indian sub-continent (Srinivasan *et al.*, 1999) and Australia (Clarke and Siddique 2004). The plants continue to produce flowers but fail to set pods when mean daily temperature falls below 15°C . ICRISAT has developed several breeding lines (e.g. ICCVs 88502, 88503, 88506, 88510, 88516) which are able to set pods at lower temperature (mean daily temperature between 12°C to 15°C). It has been established that the pollens of chilling tolerant genotypes have faster pollen tube growth than the pollens of chilling sensitive genotypes (Clarke and Siddique, 2004). A pollen selection method has been developed and successfully applied for transferring cold tolerance from ICCV 88516 to the popular variety Amethyst in Australia (Clarke *et al.*, 2004). Efforts have also been made to identify molecular markers for chilling tolerance. RFLP markers were identified and converted to SCAR markers. These markers were successful in the selection of chilling tolerant progeny derived from a cross between Amethyst and ICCV 88516. However, these markers were not effective in other crosses (Millan *et al.*, 2005).

Soil salinity: Soil salinity is a major constraint to chickpea productivity in many parts of India, Pakistan, Iran, West Asia, North Africa and Australia. The chickpea plants show reduction in growth, high anthocynin pigmentation of foliage in *desi* type and yellowing of foliage in *kabuli* type, reduction in biomass, seed size and grain yield. Genotypes that can tolerate moderate levels of salinity have been identified in India (Dua and Sharma, 1995), Pakistan (Asharf and Waheed, 1992) and Australia (Maliro *et al.*, 2004). A *desi* chickpea variety Karnal chana 1 (CSG 8963) that can be grown up to the salinity level of 6 dS m^{-1}

has been released in India. The screening of limited number of accessions of wild *Cicer* species did not identify any tolerant source for soil salinity (Maliro *et al.*, 2004).

Because earlier screening for salinity tolerance in chickpea had always included a fairly limited number of accessions, compare to the availability of genetic resources, ICRISAT scientists have recently screened 252 germplasm accessions (including 211 accessions of mini-core collection) and breeding lines/cultivars for salinity tolerance using pot screening method. Wide variation was observed for salinity tolerance (Serraj *et al.*, 2004b). The experiment is being repeated during 2004/05 crop season for confirmation of results, including the material previously identified as tolerant by Dua and Sharma (1995). Nevertheless, these preliminary results have renewed interest in research on salinity tolerance in chickpea. Prospects are also there to screen the same material for tolerance to sodicity. There seems to be a good prospect to use the salinity tolerant chickpea materials in rice fallows in coastal saline areas.

BIOTIC STRESSES

Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *ciceri*

It is the most important root disease of chickpea, particularly in the semi-arid tropics (SAT) where the chickpea growing season is dry and warm. Seven races of the fungus have so far been reported – races 1 to 4 from India (Haware and Nene 1982a), races 0 and 5 from Spain (Jimenez – Diaz *et al.*, 1989) and race 6 from California, USA (Phillips 1988). Availability of simple and effective field screening technique and many highly resistant sources with disease score of 1 (on 1 to 9 scale, going from high to low resistance) have led to excellent progress in breeding FW resistant cultivars, particularly for races 1 to 4. Germplasm lines/cultivars are available that possess resistance to multiple races. For example, WR 315 is resistant to all races, except race 3; and JG 74 is resistant to all races, except races 2 and 5 (Haware 1997). The resistance to FW seems to be very stable.

Millan *et al.* (2006) has recently reviewed results of various studies on genetics of FW resistance and mapping of FW resistance genes. Three genes have been reported each for race 1 and race 2, two for race 4 and one each for race 0 and race 5. The genes *foc1*, *foc3*, *foc4* and *foc5* involved in resistances to races 1, 3, 4 and 5, respectively are organized in two clusters on linkage group (LG) II, whereas the gene *foc0₁*, for resistance to race 0 is located on LG 5. SSR markers closely linked with these FW resistance genes have been identified and can be used for pyramiding resistance genes for various races.

Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass)

It is the most devastating foliar disease of chickpea globally, particularly in Pakistan, northwest India, west and central Asia, North Africa, North America and Australia. It occurs mainly in areas where cool, cloudy and humid weather occurs during the crop season. The AB pathogen is highly variable. Random mating may occur between different pathotypes that coexist in the same field and carry different mating type alleles (Brave *et al.*, 2003). Genetic recombination in fungus may contribute to genotypic diversity and provide the fungus with an additional means to adapt to newly introduced resistant

cultivar. The progress on AB research in chickpea has recently been reviewed by Pande *et al.* (2005).

Several germplasm accessions with moderate resistance to AB have been identified (Reddy and Singh 1984). A scoring scale of 1 to 9 (going from high to low resistance) is generally used for disease scoring. Four lines (ILC 72, ILC 191, ILC 3279, ILC 3856) showed resistance in eight countries (Singh *et al.*, 1984) and five lines (ICC 4475, ICC 6328, ICC 12004, ILC 200, ILC 6482) were resistant to six races (Singh and Reddy 1993). Several varieties with moderate resistance to AB have been released in many countries from the breeding material supplied by ICARDA and ICRISAT or developed by their own breeding programs.

There are variable reports on number of genes involved in AB resistance. This is probably because of variation in the fungal isolates and cultivars used. The recent studies on RIL populations indicate that several major and minor QTLs are involved in AB resistance (reviewed by Millan *et al.*, 2006). Thus, it should be possible to enhance the level of resistance by accumulating resistance genes from different sources. Some of the progenies derived from multiple crosses at ICRISAT have shown higher level (score 3 to 4) of AB resistance to multiple isolates. Millan *et al.* (2006) has recently reviewed progress on identification of markers for AB resistance QTLs. SSR markers are now available for all major QTLs involved in AB resistance and it seems feasible to use MAS for pyramiding AB resistance genes.

Botrytis gray mold (BGM) caused by *Botrytis cinerea* Pres.

It is the second most important foliar disease of chickpea, particularly important in northern India, Nepal, Bangladesh, Pakistan and Australia. It is considered to be the major cause for decline in chickpea area in Nepal and Bangladesh. The BGM fungus is necrotrophic and has extensive host range, high variability and wide adaptability. Though it infects all aerial parts of the plant, flowers are the most severely affected plant part leading to poor or no pod set. There are reports that indicate existence of different pathotypes of *B. cinerea* (Singh and Bhan 1986, Rewal and Grewal 1989). A dominant gene for resistance was identified in some crosses and two genes with epistatic interaction in other crosses (Chaturvedi *et al.*, 1985, Rewal and Grewal 1989).

Over 6000 chickpea accessions were screened at ICRISAT for resistance to BGM, but none showed high level of resistance (Haware and Nene 1982b, Haware and McDonald 1993). Some accessions with erect plant type, such as ICCL 87322 and ICCV 88510, were found to be less affected by the disease (Haware and McDonald 1993). It appears that erect plant type helps in air circulation that reduces buildup of humidity and spread of disease. Of the 36 accessions of annual wild *Cicer* species screened at ICRISAT under controlled environment growth room, only three accessions of *C. bijugum* (ICCW 41, 42 and 91) were found resistant (Haware *et al.*, 1992). ICCW 41 and 42 also showed resistance to AB.

Poygalactoseuronases are the key enzymes in the invasion of plant tissues by many facultative fungal pathogens. A polygalacturonase inhibitory protein (PGIP) identified from immature raspberry fruit was found effective against endo-poygalactoseuronases produced constitutively by *B. cinerea* (Johnston *et al.*, 1993). At ICRISAT, PGIP gene and other antifungal genes such as chitinases and glucanases are being introduced into chickpea for resistance to BGM and other fungal diseases. Efforts are also being made to identify and

clone tissue-specific promoters for more controlled expression of these potential transgenes.

Pod borer (*Helicoverpa armigera* Hübner)

It is the most important insect-pest of chickpea globally. The insect is highly polyphagous and the sources with high level of resistance are not available in chickpea germplasm. Thus, it has not been possible to breed varieties which can provide adequate level of resistance to this pest.

Extensive efforts have been made at ICRISAT and by Indian NARS to identify germplasm resistance to this pest. The grading for resistance is usually done on 1 to 9 scale (going from high to low resistance). Several germplasm accessions/breeding lines/cultivars have been identified that show low to moderate resistance (see Sharma *et al.*, 2003 for a recent review). Over 160 accessions of annual wild *Cicer* species have been screened at ICRISAT for *Helicoverpa* resistance. On 21 of these accessions the larval growth was slow and this phenomenon of antibiosis was unique to the wild species (Sharma *et al.*, 2002). Efforts are being made to combine the non-preference (antixenosis) mechanism of resistance identified in the cultigen (e.g. ICC 506 EB) and antibiosis mechanism of resistance identified in *C. reticulatum*. The preliminary screening of some of perennial wild *Cicer* species revealed that *C. microphyllum* and *C. canariense* had pod borer rating as low as 1.0, while *C. judaicum*, reported earlier as a source of resistance, had a damage rating of 4.0, and cultivated chickpea genotypes had leaf and pod damage rating of 8.5 and 9.0 (Rai *et al.*, 2004). Thus, these two species offer the best source of *Helicoverpa* resistance in chickpea. However, these are not accessible currently due to crossability barrier with the cultigen.

Presently, transgenic resistance is being considered as the most potential approach for enhancing chickpea resistance to *Helicoverpa*. Transgenics using *Cry1Ab*, *Cry1Ac* and *SbT1* have already been developed at ICRISAT and are being subjected to insect bioassays. The first ever-contained field trial of chickpea transgenics is being conducted during 2004/05 crop season using T₃ plants carrying *Cry1Ab* gene. The results of this trial are eagerly awaited.

FUTURE PROSPECTS

The global importance of chickpea has increased in the recent years, primarily due to expansion of its cultivation in new areas (Australia and Canada), increase in its global trade, increasing preference for vegetable protein, and increased availability of chickpea preparations in the restaurants of western countries. There has been an increase in the number of advanced research centers working on chickpea and publications on chickpea in international journals. It is particularly so for biotechnological research. Formation of an International Chickpea Genomics Consortium (www.icgc.wsu.edu) is another evidence of increased global interest in chickpea research.

The recent years have witnessed rapid progress in molecular mapping of agronomically important traits (reviewed by Ahmad *et al.*, 2005 and Millan *et al.*, 2006). The molecular markers identified for FW and AB resistance genes are ready to be used in breeding programs for pyramiding of resistance genes through MAS. Excellent progress has been

made in development of protocols for *in vitro* regeneration and transformation of chickpea. Transgenics for *Helicoverpa* pod borer are already under contained field testing and others are in pipeline. Introgression of transgenes from transgenics to other cultivars can easily be achieved through conventional breeding approaches.

The Generation Challenge Program of the CGIAR has included chickpea as one of its target crops. It is focusing on comparative genomics and association mapping for identification and cloning of candidate genes and moving them into targeted crops/cultivars using transgenic technology. This approach will also be useful in accessing useful genes from wild *Cicer* species that currently cannot be crossed with the cultivated species.

It is expected that applications of biotechnological approaches in combination with the conventional approaches would provide rapid progress in chickpea improvement in the coming years. The scientific community should make sure that the ultimate goal of its research is not more information and greater knowledge, but its application to the needs of farmers.

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