Improving Drought-Avoidance Root Traits in Chickpea (Cicer arietinum L.) -Current Status of Research at ICRISAT

Pooran M. Gaur, Lakshmanan Krishnamurthy and Junichi Kashiwagi

(International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India)

Abstract: Chickpea (Cicer arietinum L.), an important food legume grown in the semi-arid tropical and Mediterranean regions, suffers substantial yield loss due to drought at the end of the growing season (terminal drought), as the crop is largely grown rainfed in post-rainy season on progressively receding soil moisture conditions. Root traits have been identified to postpone dehydration (drought avoidance hereafter) under moisture stress. The root length density (RLD) in the relatively shallow soil layers and the maximum root depth (RDp) were found to positively influence the seed yield under terminal drought environments. Considerable progress has been made to improve the methodology for sampling and analysis of roots. Using a PVC cylinder technique, the mini-core collection (n=211) of chickpea germplasm was evaluated for a number of root traits, including root biomass, RLD and RDp. A few germplasm accessions were identified to have a more prolific root system than the previously identified germplasm line ICC 4958, the best-known source of high root biomass. The germplasm accession ICC 8261 was identified to have the best combination of both RLD and RDp. Molecular markers have been identified for one major quantitative trait locus (QTL) that accounts for about one-third of the variation in root biomass (as measured by total root dry matter) and RDp from study of recombinant inbred lines (RILs) derived from a cross between ICC 4958 and Annigeri. New RIL populations, developed from two other crosses (ICC 8261 × ICC 283 and ICC 4958 × ICC 1882) involving parents having larger variation for root traits than between Annigeri and ICC 4958, are being studied to identify additional QTLs for root traits. Marker-assisted breeding for improvement of root traits in chickpea is expected to promote the development of varieties with greater drought avoidance.

Key words: Chickpea, Cicer arietinum L., Drought avoidance, Root traits, Terminal drought.

Chickpea (Cicer arietinum L.) is a self-pollinated diploid (2n=2x=16) food legume originating in southeastern Turkey (Ladizinsky and Adler, 1976) and subsequently spread to India and Europe (Singh and Auckland, 1975), where it is usually grown in arid or semi-arid climates. There are two types of chickpea, desi (colored seed) and kabuli (white or beige colored seed). The desi type covers about 85% of the global chickpea area and is predominantly grown in South and East Asia, Iran, Ethiopia and Australia, while the kabuli type is grown mostly in the Mediterranean regions, West Asia, North Africa and North America.

Chickpea ranks second in area and third in production among food legumes. In 2005, the global chickpea area was 11.2 million ha, the production was 9.2 million tons and the average yield was 818 kg ha⁻¹ (FAOSTAT, 2006). Though chickpea is grown in about 50 countries, 95% of its area is in the developing countries where South Asia alone accounts for about 71% of the global area. Most of the chickpea production is consumed locally and the global trade is about 12% of the total production. Thus, chickpea is an important source of protein for millions of people in developing countries. In addition to having a high protein content (20-22%), chickpea is rich in fiber and minerals (phosphorus, calcium, magnesium, iron and zinc) and its lipid fraction is high in unsaturated fatty acids (William and Singh, 1987). Chickpea contains larger amounts of carotenoids such as β-carotene than genetically-engineered ‘golden rice’ (Abbo et al., 2005). Chickpea is also used as a protein-rich animal feed and the vegetative biomass is used as fodder.

Chickpea can fix up to 140 kg nitrogen ha⁻¹ and meet up to 80% of its nitrogen requirement from symbiotic nitrogen fixation (Saraf et al., 1998). It leaves behind a substantial amount of nitrogen through crop residue for subsequent crops and adds much needed organic matter to maintain and improve soil health, long-term fertility and sustainability of the ecosystems.

Terminal drought (the soil moisture stress that occurs at the pod filling and seed development stage of the crop with increasing severity at the end of season) is a major constraint to chickpea production in over 80% of the global chickpea area, as the crop is largely grown rainfed in post-rainy season. Efforts to breed drought tolerant varieties in the past have not been rewarding because of imperfect understanding of drought manifestation and using yield as an empirical selection criterion (Saxena, 2003). Several
studies in recent years have focused on identification of morphological and physiological traits associated with drought tolerance. Root traits, such as root depth and root biomass, have been identified as the most promising plant traits in chickpea for terminal drought tolerance, as these help in greater extraction of available soil moisture. Most of the research on root traits of chickpea has been conducted at ICRISAT and some in partnership with the National Agricultural Research System (NARS) scientists in different countries. This article provides a brief review of the research progress and highlights future prospects of improving root traits for enhancing drought avoidance in chickpea. We hope that the article will be useful to researchers working on improving drought tolerance in legumes, particularly chickpea.

1. Chickpea growing environments

Chickpea is predominantly grown as a rainfed crop on residual soil moisture stored during the previous rainy season with very less or no rainfall during the growing season. For example, in the arid and semi-arid tropical environments of South and Southeast Asia, chickpea is grown in winter season after the rainy season. Similarly in the Mediterranean environments, it is grown in spring on stored soil moisture from winter and early spring rainfall. In both environments, the soil moisture recedes to deeper soil layers with the advancement in crop growth and the crop experiences increasing soil moisture deficit at the critical stage of pod filling and seed development (Saxena, 1984; Siddique et al., 2000). Patancheru (altitude: 545 m above the mean sea level, latitude: 17°27’N, longitude: 78°28’E) in southern India where ICRISAT’s headquarter is located has a typically short chickpea growing season because the soil moisture keeps depleting progressively under the high atmospheric evaporative demand with normally little or no precipitation during the crop season (Fig. 1). The extent of terminal drought stress varies depending on previous rainfall, atmospheric evaporative demand, and soil characteristics such as type, depth, structure, and texture. Terminal drought is globally the most serious constraint to chickpea productivity. It is estimated that if the soil water stress is alleviated, chickpea production could be improved up to 50% that is equivalent to approximately 900 million US dollars (Ryan, 1997). Extensive research efforts have been made to reduce the yield loss of chickpea under the drought environments. However, many issues related to drought are yet to be resolved since drought is a highly complex phenomenon.

2. Mechanisms for drought escape/tolerance

Plants are known to have different mechanisms to adjust to water stress conditions. Plant breeders generally categorize these mechanisms into three categories—(1) drought escape, (2) drought avoidance, and (3) drought tolerance. However, some physiologists suggest that these mechanisms should be categorized as (1) drought escape, (2) dehydration postponement, and (3) dehydration tolerance because water deficit affects the hydration of the plant (Kramer, 1980; Turner, 1986; Blum, 1988). Though the focus of this review is on drought-avoidance root traits, a brief account of research progress on other plant mechanisms for adaptation of chickpea to drought environments is given here.

1. Drought escape

Early phenology (early flowering, early podding
and early maturity) is the most important mechanism to escape terminal drought stress. Early flowering has been shown to be associated with high initial growth vigor in chickpea (Sabaghpour et al., 2003). The chickpea breeding program at ICRISAT has placed high emphasis on development of early maturing varieties for enhancing adaptation of chickpea to environments prone to terminal drought stress (Gaur et al., 2008). Several varieties (e.g. ICCV 2, ICCC 37, JG 11, and KAK 2) that mature in 85 to 100 days at Patancheru, as compared to the traditional varieties that mature in >110 days have been developed. The early-maturing varieties have greatly contributed to expansion of area and enhancement of productivity of chickpea in terminal drought-prone areas of peninsular India (Gaur et al., 2008) and Myanmar (Than et al., 2007). Breeding lines extra-early in maturity (75 to 80 days at Patancheru) have been developed and offer further opportunities for expanding cultivation of chickpea in new niches (Kumar and Rao, 1996; Gaur et al., 2008).

The early-maturing varieties are preferred by the farmers because of a stable yield than the late-maturing varieties. The early maturing crop, however, may not give higher yield in more favorable seasons as it can not accumulate enough total plant biomass due to reduced total photosynthetic period compared to the relatively longer maturing varieties. Thus, there is a need to match the crop duration with the available length of the crop season for realizing high yield.

(2) Dehydration postponement (drought avoidance)

The process whereby plants maintain a high water potential or turgor pressure under soil water deficit conditions is called dehydration postponement (Turner, 2003). This can be achieved by water uptake by the roots from deeper soil layers, by reducing water loss or by osmotic adjustment (Turner and Jones, 1980; Turner, 1986). The role of root traits, such as root depth and root vigor, in extraction of water from deeper soil layers under depleting soil moisture conditions is well recognized and will be discussed later in greater detail.

The water loss can be reduced through stomata conductance or by reduction in leaf area due to leaf shedding or change in leaf morphology (e.g. few leaflets, tiny leaves). Differences in stomatal conductance of chickpea leaf in response to water potential have been reported (Lawn, 1982; Muchow, 1985). Abscisic acid (ABA) has been shown to have an important role in stomatal conductance in lupin (Gallardo et al., 1994). Nayyar et al. (2005) found higher ABA contents in the wild Cicer species Cicer reticulatum than in the cultivated species under water stress.

Reduction in leaf area is expected to reduce water loss. Saxena (2003) reported two chickpea accessions, ICC 5680 and ICC 10448, with a smaller leaf area. ICC 5680 has fewer leaflets, while ICC 10448 has narrow leaflets. The fewer leaflet trait in ICC 5680 reduced transpiration loss of water by 30% compared to ICC 4958 in experiments conducted under controlled environmental facilities at ICRISAT (Saxena, 2003). Breeding lines that combined the large root traits of ICC 4958 and few leaflet trait of ICC 5680 have been developed (Saxena, 2003). The data on the response of these lines to drought tolerance is not available. In another study, Toker and Canci (2007) did not observe any advantage of multipinnate or tiny leaf types in drought tolerance.

In osmotic adjustment (OA), solutes are accumulated in the cell in response to water deficit. This accumulation of solutes in the cell reduces its water potential attracting movement of water into the cell leading to greater extraction of water from the soil, as observed in wheat (Morgan, 1983), sorghum (Basnayake et al., 1996) and barley (Gonzalez et al., 1999). Although OA has been reported to be an important trait for drought tolerance in some cereal crops, e.g. wheat (Morgan et al., 1986) and sorghum (Tangpremsri et al., 1995), there are variable reports on the association of OA with grain yield in chickpea. Some studies have shown an association between OA and seed yield under water stress conditions (Morgan et al., 1991; Moinuddin and Khanna-Chopra, 2004), while some studies found inconsistent or no relationship (Singh et al., 1990; Leport et al., 1999). A recent study conducted at multiple locations in India and Australia concluded that phenotypic expression of OA is not stable and it can not be considered as a selectable drought tolerance trait in chickpea breeding programs (Turner et al., 2006)

(3) Dehydration tolerance (drought tolerance)

Dehydration tolerance refers to the ability of cells to continue metabolism at a low leaf water status (Turner et al., 2003). Membrane injury occurs when dehydration reaches a critical point. Though electrolyte leakage from the cell is a measure of cell injury (Nayyar et al., 2005), the relationship between electrolyte leakage and crop performance under water-limited conditions has not been demonstrated (Blum and Ebercorn, 1981; Blum, 1988).

Proline accumulation in the cytosol has been reported to occur in many legumes, including soybean, faba bean, field pea, and common bean, as a response to water deficits (Hanson and Nelson, 1980). However, selection for lines with high proline accumulation suggested that proline was not a selection criterion for improved drought tolerance (Hanson et al., 1979; Hanson and Hitz, 1982). Transgenic chickpea plants over-expressing the gene encoding Delta 1-pyrroline-5-carboxylate synthetase (P5CS), the enzyme involved in proline biosynthesis, have been produced (ICRISAT, 2005). The transgenic plants did not differ significantly
from the wild-type in transpiration efficiency. However, wide differences were observed for total transpirable soil water and stomatal conductance, which need further investigation.

3. Grain yield under soil moisture-stress conditions

Analytically, grain yield (YLD) under drought environments can be described by the following expression (Passioura, 1977; Fischer, 1981):

\[ \text{YLD} = \text{Transpiration (T)} \times \text{Transpiration Efficiency (TE)} \times \text{Harvest index (HI)} \]

Thus, improvement in any one or the combinations of the above components is expected to improve grain yield under drought. Improvement in harvest index, the third component in the above expression, is believed to be relatively less cumbersome and therefore can be dealt with at the last stage of breeding and selection. Hence, improvement efforts for the components transpiration and transpiration efficiency need to be attended on a priority basis. The total shoot biomass can be increased either by increasing transpiration or transpiration efficiency. Under water-limited receding soil moisture conditions, the root system that can extract relatively more water from deeper soils and/or absorb water relatively rapidly (thereby maximizing transpiration over evaporation) can increase total water transpired. Increased transpiration is not only expected to increase the total biomass productivity but also improves the harvest index (Passioura, 1977, 1994; Kashiwagi et al., 2006). In several crops, such as common bean (White and Castillo, 1990), groundnuts (Wright et al., 1991) and soybean (Cortes and Sinclair, 1986), deep rooting has been demonstrated to have positive effects on seed yield through improved transpiration. This suggests that the theoretical approach to increase transpiration through better soil water uptake by root system improvement could be successfully employed in any drought tolerance breeding program. However, breeding efforts to improve seed yield or shoot biomass under drought environments through root system improvement have been limited due to lack of techniques that would allow large-scale screening of genotypes, limited information on genetic variability in root traits, and poor understanding of the genetics of root attributes.

4. Current research status on drought avoidance root traits in chickpea

(1) Importance of root traits in drought avoidance

The yields of chickpea genotypes under rainfed and irrigated conditions were compared at ICRISAT to gather information on yield under drought conditions and potential yields (Saxena, 2003). More than 1500 chickpea germplasms plus released varieties were subjected to field screening (Saxena, 1987, 2003). Some genotypes (e.g. Annigeri, ICC 4958, ICC 10448, ICC 5680 and JG 62) were identified to have higher drought tolerance indices (standard residuals calculated after removing the early flowering and yield potential effects through a regression approach; see Saxena, 1987), although each had a different trait or mechanism for coping with terminal drought. One genotype, ICC 4958, that exhibited the best performance not only in field trials at ICRISAT but also at several other locations in India and in the Mediterranean type climate at Syria, had higher root biomass (ICARDA, 1989; Saxena et al., 1993; Krishnamurthy et al., 1996; Ali et al., 1999, 2005). Subsequently, in a field experiment at ICRISAT with 12 diverse chickpea germplasms, including ICC 4958, it was shown that a prolific root system, especially at the 15-30 cm soil depth, contributed positively to the seed yield under moderate terminal drought intensity and a deeper root system was shown to contribute to improved yield under severe terminal drought conditions (Kashiwagi et al., 2006). Also in a cool-temperate sub-humid climate of New Zealand, the importance of surface soil horizons (0-30 cm) in providing major water requirements of kabuli chickpeas and the ability of chickpea to draw water from depths below 60-cm have been clearly shown (Anwar et al., 2003). The advantage of a deep root system towards drought tolerance was also substantiated in soybeans (Kaspar et al., 1978), common beans (Sponchiado et al., 1989) and chickpea (Silim and Saxena, 1993). Some major root attributes such as greater efficiency in water absorption per unit root length density, ability to change the rooting pattern across soil depths to efficiently access the available soil moisture and the ability to produce a larger root surface area per unit root biomass seem to make chickpea the best choice for the dryland cropping systems compared to other legumes or cereals (Thomas et al., 1995; Ali et al., 2002; Tilahun and Schubert, 2003; Benjamin and Nielsen, 2006). Root length density of chickpea has been shown to be substantially lower than that of barley but, absorbed water more efficiently than barley plants (Thomas et al., 1995). The difference in water use between these species was a function of root hydraulic conductivity, which is governed by the diameter and the distribution of the meta-xylem vessels (Hamblin and Tennant, 1987). Chickpeas have the ability to change their root distribution across soil depths depending on the soil moisture availability. The chickpea root system at the mid-pod fill stage has been shown to be two to three times greater in the surface soil layer (0–15 cm) alone when irrigated, matching the irrigation effect of two to three times greater shoot biomass productivity at maturity. Whereas the proportion of root length density distributed at deeper soil layers (115–120 cm) was shown to be higher under receding soil moisture
conditions (Ali et al., 2002). In another comparison, chickpea and field pea have been found to have a greater proportion of their root system deeper in the soil profile under dryland conditions compared to irrigated conditions (Benjamin and Nielsen, 2006) while soybean was found to have a similar proportion of roots distributed across depths irrespective of irrigation treatments. Additionally, chickpea was found to possess a higher root surface area to root weight ratio compared to field pea or soybean (Benjamin and Nielsen, 2006). These results suggest that chickpeas are better equipped towards tolerance to drought stress and further improvement of root traits would be one of the promising approaches to improve the drought avoidance of chickpea under the terminal drought environments.

(2) Refinement of root research methodology

Conducting research on root systems in a field condition is very laborious, expensive and time-consuming (Subbarao et al., 1995). ICRISAT has established a modified monolith method (Serraj et al., 2004) which is fairly reliable and allows systematic field root extraction at a root sampling rate of approximately 3.3 profiles worker$^{-1}$ day$^{-1}$. Although this method is fairly reliable, it cannot be employed for large scale screening of genotypes. The pot-culture method is less cumbersome but rooting profile can not be estimated in shallow pot grown plants. Thus, extensive efforts have been made at ICRISAT to optimize a PVC cylinder culture system as an alternative method that allows screening of large number of genotypes. Tall PVC cylinder system was optimized for use to estimate the chickpea root growth since the rooting profile can not be estimated in shallow pot-grown plants. When the plants were grown in PVC cylinders 18 cm in diameter and 120 cm in height, filled with a sand-vertisol mixture containing a soil moisture equivalent to 70% field capacity, the extracted root biomass was demonstrated to be significantly correlated to the ones extracted from the field ($r=0.62$, $p<0.05$) (Kashiwagi et al., 2006). With this system, the sampling efficiency could be improved dramatically up to about 25 profiles worker$^{-1}$ day$^{-1}$. In addition, the availability of image capturing and analysis system has facilitated rapid conversion of a large number of the intact root samples into digitized images (>150 samples day$^{-1}$) and the WINRHIZO (Regent Instruments INC, Canada) software allowed conversion of more than 500 images day$^{-1}$ into digital data of root attributes. These advancements have considerably increased efficiency of root sampling and analysis. Needless to say, availability of substantially simpler methods of root growth estimations would facilitate root research. Root growth estimations at early vegetative growth stages (Canci et al., 2004) may be of limited use considering the growth stage × genotype interaction for root growth (Krishnamurthy et al., 1996)

(3) Genetic variability in root traits

Efforts have been made to assess genetic variability in root traits of the chickpea germplasm. A mini-core collection (n=211) of chickpea germplasms was developed at ICRISAT (Upadhyaya and Ortiz, 2001), and it was evaluated for root traits using the cylinder culture system subsequently. Large and significant variation was found among the accessions of the mini-core collection for root length density (RLD), root dry weight (RDW), rooting depth (RDp) and root to total plant weight ratio (R/T) (Kashiwagi et al., 2005). The significant genotype × season interaction that occurred for RLD and R/T in this study was found to be of non-crossover type. This was assessed by employing a rank
correlation between the accession means of the two seasons. The accession ICC 4958, earlier identified to have a large root system, was among the top ranking genotypes for a prolific root system. In addition, an accession, ICC 8261 was identified as the one with the most prolific and deep root system among the chickpea mini-core collection (Fig. 2). In one season, the root traits of 10 accessions of annual wild Cicer species were also evaluated. The root systems of wild relatives were found to be smaller than Cicer arietinum, except for the most closely related species C. reticulatum which has a root system more or less comparable to that of the average root system of C. arietinum.

A study was conducted to estimate the gene effects on root traits. Two crosses, ICC 283 (smaller roots) × ICC 8261 (larger roots) and ICC 4958 (larger roots) × ICC 1882 (smaller roots), were made. The parents (P1, P2) and the F1, F2, BC1, and BC2 generations from these crosses were used for estimation of gene effects through generation mean analysis. In both the crosses, the additive and additive × additive interaction effects played an important role in governing the root length density and root dry weight. The direction of the additive gene effects was consistent and towards increasing the root growth. Generating larger populations and delaying selections to later generations were proposed to exploit additive × additive gene interaction for improving the root system of chickpea (Kashiyagi et al., 2008).

5. Molecular mapping of QTLs for drought avoidance root traits

Despite the importance of root traits in drought avoidance and availability of germplasms with prolific root systems, the breeding efforts to improve root traits have been negligible. This is because of the laborious, time-consuming and destructive methods involved in root studies. Molecular markers linked to major quantitative trait loci (QTLs) for root traits can greatly facilitate marker-assisted selection (MAS) for root traits in segregating generations. Over 500 simple sequence repeat (SSR) markers have been developed in chickpea (Hüttel et al., 1999; Winter et al., 1999; Lichtenzweig et al., 2005) and the chickpea genome map is rapidly expanding (Millan et al., 2006).

ICRISAT has generated a set of over 2800 chickpea expressed sequence tags (ESTs) from a library constructed after subtractive suppressive hybridization (SSH) of root tissue from ICC 4958 and Annigeri to isolate and characterize root-specific genes differentially expressed between these genotypes (Jayashree et al., 2005; Buhariwalla et al., 2006). This database provides researchers in legume genomics with a major new resource for data mining associated with root traits and drought tolerance.

A set of 257 recombinant inbred lines (RILs) from Annigeri × ICC 4958 cross was developed at ICRISAT and characterized for root traits (Serraj et al., 2004) and SSR markers. A SSR marker, TAA 170, was identified for a major QTL that accounted for 33.1% of the variation for root weight and root length (Chandra et al., 2004). Based on the screening of mini-core collection, parents more genetically and phenotypically distant were identified for development of new mapping populations. These include ICC 8261 and ICC 4958 for a large root system and ICC 283 and ICC 1882 for a small root system. Two crosses (ICCV 283 × ICC 8261, ICC 4958 × ICC 1882) were made and about 260 RILs were developed from each cross. These two mapping populations have been phenotyped in 2005 (ICC 4958 × ICC 1882) and in 2006 (ICC 283 × ICC 8261). Genotyping study is under progress to generate adequate number of markers to identify additional QTLs for root traits.

6. Future prospects

The recent advancements in refinement of methodologies for root studies and identification of the large variation in root traits of the germplasm have increased interest of researchers in exploiting root traits for improving drought tolerance in chickpea. MAS for root traits is expected to greatly facilitate breeding for root traits. There has been considerable progress in development of SSR markers and expansion of genome map of chickpea in recent years. Molecular markers have already been identified for a major QTL and efforts are being made to map additional QTLs for root traits. The marker-assisted breeding for root traits in chickpea is likely to begin soon. However, we should keep in mind that the effectiveness of root traits in improving drought avoidance would vary depending on growth environments, e.g., soil type, moisture status of the soil, soil compaction, etc. There is a need to characterize the drought environments and identify suitable mechanism(s) of drought tolerance for each specific environment. Application of remote sensing technology would be promising in characterization of drought environments (Thenkabail et al., 2004).

Although the importance of root traits in drought avoidance is well-recognized, other plant mechanisms for coping with drought stress are not well understood in chickpea. It is important to conduct multi-locational field trials to identify other traits involved in drought tolerance and exploit them in combination with root traits. Once the relevant traits are identified, the genes controlling these traits can be tagged with molecular markers. MAS will be very useful in combining different drought tolerance traits to develop cultivars that can provide drought tolerance in a range of drought environments.

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