Chapter 10
Genomics and Physiological Approaches for Root Trait Breeding to Improve Drought Tolerance in Chickpea (*Cicer arietinum* L.)

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10.1 Chickpea Crop

Chickpea is a valuable agricultural crop of South Asia and the third most important pulse crop in the world after dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum*

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sativum L.). Cultivated chickpea, *Cicer arietinum* L., is a self pollinated, diploid ($2n = 2x = 16$) annual pulse crop with a genome size of 750 Mbp (Arumuganathan and Earle 1991). There are two types of chickpea: *desi* (brown colored small seed) and *kabuli* (white or beige colored large seed). *Desi* type covers about 85% of global chickpea area and is predominantly grown in South and East Asia, Iran, Ethiopia, and Australia, and the *kabuli* type is grown mostly in the countries of the Mediterranean regions, West Asia, North Africa, and North America. The wild ancestor of domesticated chickpea is *Cicer reticulatum*. Chickpea originated in southeastern Anatolia (Turkey) and was traditionally cultivated in Asia, the Mediterranean, the Middle East, and northern Africa (Ladizinsky and Adler 1976). In contemporary times, chickpea has become popular throughout the temperate regions in countries such as Mexico, Canada, and Australia (Duke 1981).

Chickpea ranks third among pulses, fifth among grain legumes, and 15th among grain crops of the world. In 2006, the world chickpea cultivation area was 10.7 Mha with over 8 Mha grown in India, Pakistan, and Iran, with a further 1 Mha grown in other countries of Asia, the Middle East, and Canada. Total production was 8.4 Mt, and the average yield was 772 kg/ha (FAOSTAT 2006). Although chickpea is cultivated in about 50 countries, 95% of its area is in the developing countries where South Asia alone covers almost 71% of the world chickpea harvested area. Most of the chickpea harvested is consumed locally and the global trade is about 12% of the total production. The global demand for chickpea is projected to be 11.1 Mt in 2010. Under optimum growing conditions, the yield potential of chickpea is 6 t/ha (Singh 1987), which is much higher than the current global yield average of ~0.8 t/ha (Ahmad et al. 2005).

10.2 Drought Stress in Chickpea

The main constraints in chickpea production are the abiotic stresses such as drought, heat, cold, and high-salinity and the biotic stresses such as *Ascochyta* blight, *Fusarium* wilt, and the pod borer. The estimated collective yield losses due to abiotic stresses (6.4 Mt) are higher than that of the biotic stresses (4.8 Mt) (Ryan 1997). In the order of importance, drought, cold, and salinity are the three main abiotic stresses that affect chickpea growth and productivity worldwide (Croser et al. 2003). Drought stress alone causes a 40–50% reduction in yield globally (Ahmad et al. 2005). It is estimated that if the yield loss due to drought stress is alleviated, chickpea production could be improved up to 50%, equivalent to approximately US$ 900 million (Ryan 1997).

As 90% of chickpea crops are cultivated under rainfed conditions, drought is of major concern (Kumar and Abbo 2001), with terminal drought as the major constraint limiting productivity. Terminal drought stress is typical of the post-rainy season crop in the semiarid tropical regions, where the crop grows and matures on a progressively receding soil moisture profile (Ludlow and Muchow 1990; Krishnamurthy et al. 1999), and the intensity of terminal drought varies depending

on previous rainfall, atmospheric evaporative demand, and soil characteristics such as type, depth, structure, and texture. In the arid and semiarid tropics of South and Southeast Asia, chickpea is grown in the winter season immediately after the end of the rainy season. Similarly in the Mediterranean environments, it is grown in spring on stored soil moisture from the winter and early spring rainfall. In both the 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).

10.3 Strategies to Tackle Drought Stress

Two main strategies are envisaged to tackle drought stress in chickpea (1) developing early maturing varieties and (2) developing drought tolerant varieties (Gaur et al. 2008a, b). The breeding strategy for development of early maturing cultivars is straight forward. One of the parents used in crosses should be a well-adapted cultivar, and another parent should be an early maturity germplasm accession/cultivar. In segregating generations, plants that flower early, for instance, in 25–30 days at ICRISAT-Patancheru, are selected and their progenies are further evaluated. Selection for time to flower is effective even in early segregating generations as it is controlled by a few major genes. Early flowering is a recessive trait and controlled by a major gene *ppd* in ICC 5810 (Or et al. 1999) and by a major gene *efl-1* in ICCV 2 (Kumar and van Rheenen 2000). Early phenology (early flowering, early podding, and early maturity) is the most important mechanism to escape terminal drought stress. At ICRISAT, the chickpea breeding program 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. 2008b). Several varieties (e.g., ICCV 2, ICCC 37, JG 11, and KAK 2) have been developed that mature in 85–100 days at Patancheru, as compared to >110 days taken by the traditional varieties. The short-duration varieties have greatly contributed to the expansion of area and enhancement of productivity of chickpea in terminal drought-prone areas of peninsular India (Gaur et al. 2008b) and Myanmar (Than et al. 2007). Breeding lines have been developed, which are extra-early in maturity (75–80 days at Patancheru) and offer further opportunities for expanding cultivation of chickpea in new niches (Kumar and Rao 1996; Gaur et al. 2008b).

Early maturing varieties that escape terminal drought and heat stress were developed by the breeders and were adopted by farmers with considerable success (Kumar and Abbo 2001). However, this drought escape fixes a ceiling on the potential yield and cannot utilize the opportunities, as and when available, of extended growing periods. Therefore, for achieving high and stable yields under drought, it is necessary to develop drought-tolerant/avoiding varieties (Johansen et al. 1997). Thus, several studies in the recent years have focused on identification of morphological and physiological traits associated with drought tolerance. Cultivated chickpea (*Cicer arietinum*) has a narrow genetic base, making it difficult

for breeders to produce new elite cultivars with durable tolerance to drought stress. In addition, drought tolerance is inherited in a quantitative manner, and the direct yield or biomass assessment under field is prone to confounded environmental effects. Therefore, selection of drought-tolerant plants in the field becomes difficult. Recent advances in genomics can assist crop improvement efforts (Varshney et al. 2005). In fact, marker-assisted selection (MAS) approach has been successfully deployed in developing improved varieties/lines/hybrids in several crop species (see Varshney et al. 2006, 2010). Quantifying the effects of drought stresses, however, involves measurement of various factors like days to flowering and maturity, early shoot growth vigor, yield, shoot biomass production, rooting depth, root length density, root to shoot ratio, total transpiration, and transpiration efficiency. Therefore, developing molecular markers for drought tolerance *per se* is a difficult task. Dissection of such complex traits into components or identification of highly related surrogate traits can enhance the heritability of such traits and facilitate development of molecular markers associated with each of such traits.

10.3.1 Targeting Root Traits for Drought Tolerance

Root traits, such as root depth and root proliferation, have been identified as the most promising traits in chickpea for terminal drought tolerance, as these help in greater extraction of available soil moisture. As these traits are quantifiable under drought stress conditions, it seems feasible to develop molecular markers for these traits and thereby can be used to screen the germplasm for drought tolerance.

One of the important physiological reasons to target root traits under the water-limiting environments is the capability of root systems to absorb relatively more water from deeper soils and/or absorb water relatively rapidly. Chickpea is a crop that is often grown in deeper and heavier soils such as vertisols under progressively receding soil moisture with little precipitation during the crop growth period. Heavier soils are characterized with soil cracking as a consequence of shrinking when dry. These soil cracks aid in enhancing soil evaporation from deeper soil layers, more so under increasing atmospheric evaporative demand coinciding with the reproductive growth stage of the crop. Therefore, it becomes necessary to maximize transpiration over evaporation (Johansen et al. 1994) and to enhance crop growth before the water is lost in cracking heavier soils. More prolific roots at the early stages of growth have been shown to be advantageous for such maximization as the root length density (RLD) values recorded in chickpea were suboptimal (Krishnamurthy et al. 1996; Kashiwagi et al. 2006). However, root prolificacy may not be expected to maximize transpiration in environments where the evaporative demands are too extreme, and also this trait may not help under environments characterized with excessive vegetative growth and poor partitioning. Similarly, deeper rooting or higher proportion of deeper root length can help in mining water from deeper soil profiles, provided the soil profiles are fully saturated in the previous rainy season or the soils are deep enough for the roots to penetrate.

Under such soil conditions, transpiration (T) gets maximized over evaporation, which can increase the total water loss under water-limited conditions. The relationship of grain yield to water-related parameters has been described by Passioura (1977) and Fischer (1981) as:

$$\text{Yield (YLD)} = \text{Transpiration (T)} \times \text{Transpiration Efficiency (TE)} \times \text{Harvest Index (HI)}.$$

The above formula indicates that the grain yield under drought could be improved through improving any one or the combinations of the above components. Also, these yield components have been shown to interact with each other. For example, the timing of water availability is shown to affect the HI. Providing small amounts of water across the growing period in comparison to the application of all the water that is required at one time was shown to favor the wheat yields through improved HI (Passioura 1977). Also, a deeper root system was found to be associated with better HI and seed yield in chickpea (Kashiwagi et al. 2006). As compared to HI, the other two factors, T and TE, can be improved by relatively less efforts. The total shoot biomass can be increased either by increasing T or TE.

In some legume crops, e.g., common bean (White and Castillo 1990), groundnuts (Wright et al. 1991), and soybean (Cortes and Sinclair 1986), deep root systems have already demonstrated to have positive effects on seed yield via improved T. These studies emphasize that the T improvement strategy for better soil moisture absorption through root systems could be applied in drought tolerance breeding program in general or at least in legumes. However, until recently, little breeding effort has been made to improve the root systems for seed yield or shoot biomass under drought environments in chickpea. The reasons include the lack of techniques that allow for large scale screening of genotypes, limited information on genetic variability in root traits, and poor understanding of the genetics of root attributes. It is also important to note that while targeting root traits in several crops has been successful to tackle drought stress in several crops, the root traits may not work in all environments.

At ICRISAT, near Patancheru in southern India (altitude: 545 m above the mean sea level, latitude: 17°27′N, longitude: 78°28′E), a team of multidisciplinary scientists has been working on root traits to improve the chickpea productivity. More than 1,500 chickpea germplasm accessions plus released varieties were evaluated under rainfed as well as irrigated field conditions at ICRISAT to gather information on the yield under terminal drought conditions and potential yields (Saxena 1987, 2003). Some genotypes, e.g., Annigeri, ICC 4958, ICC 10448, ICC 5680, and JG 62, were identified as drought-tolerant lines using a drought-tolerant index in which the effects of early flowering could be removed (Saxena 1987), although each had a different trait/mechanism to cope with the terminal drought. For example, in Annigeri and ICC 10448, narrow (lanceolate) leaves, in ICC 5680 fewer pinnules per leaf and a rapid rate of grain filling through production of twin pods at the early flowering nodes in JG 62 seem to be the mechanism contributing to

drought tolerance. The genotype, ICC 4958, showed the best performance not only at ICRISAT field trials but also at several other locations in India and in the Mediterranean climate in Syria, which was found to possess higher root biomass (ICARDA 1989; Saxena et al. 1993; Krishnamurthy et al. 1996; Ali et al. 1999, 2005). Subsequently, field experiments at ICRISAT with 12 diverse chickpea germplasm including ICC 4958 showed that a prolific root system, especially in the 15–30 cm soil depth, had positive effects on seed yield under moderate terminal drought intensity, and a deeper root system to improved yield under severe terminal drought conditions (Kashiwagi et al. 2006). The large variation in root systems within such a small group of genotypes (Fig. 10.1), and the relation between root length density (RLD) and yield under drought, suggests that an extensive and systematic screening of the chickpea germplasm might offer a promising range of variation for RLD. Furthermore, the RLD was increased under more severe stress conditions, particularly in more tolerant genotypes, and the RLD at the deeper layer was related to yield under more severe drought stress. These data suggest that the dynamics of root growth under drought conditions might be a key factor in understanding the contribution of roots to drought tolerance.

Fig. 10.1 Comparative root profiles in three chickpea genotypes. The figure shows 35-day-old plants of three chickpea genotypes, namely ICC 4958, KAK 2, and Annigeri. These plants were grown in pots in glasshouse conditions. It is evident from the figure that the root biomass for ICC 4958 is relatively higher than the other two chickpea genotypes. Higher root biomass confers high level of drought tolerance in ICC 4958 genotype.

The research on root systems under field conditions is very laborious, expensive, and time-consuming (Subbarao et al. 1995). To overcome this problem, a modified monolith method was standardized at ICRISAT (Serraj et al. 2004). This method provided systematic field root extraction at a sampling rate of 3.3 root profiles/worker/day. Although this method was fairly reliable to assess the field performance, it still did not provide an adequate sampling rate for large scale screening of genotypes. Although the less cumbersome pot-culture method was tested, the rooting profile could not be estimated in shallow pot grown plants. Thus, extensive efforts were made at ICRISAT to standardize a PVC cylinder-culture system for screening large numbers of genotypes. When the plants were grown in PVC cylinders (18 cm diameter, 120 cm height) filled with a sand–vertisol mixture containing a 70% field capacity soil moisture, the extracted root biomass was significantly correlated with the ones extracted from the field ($r = 0.62$, $p < 0.05$) (Kashiwagi et al. 2006). Moreover, the sampling efficiency of chickpea roots could be improved upto 25 profiles/worker/day. Furthermore, an image capturing and analysis system was introduced to scan the roots and convert the intact root samples into digitalized images for a large number of samples (>150 root samples/day). By using the digital image of roots, the WINRHIZO software (Regent Instruments, Inc., Canada) could generate numerical data, e.g., root length and root diameter, from more than 500 images/day.

10.3.2 Physiological Mechanisms of Root Traits

Plants take up water from soil profile using either an active or a passive water uptake pathway (Hirasawa et al. 1997). In nonstress conditions, i.e., when a plant transpires, the magnitude of active water uptake is far less than that of passive water uptake. Under severe drought conditions, however, the plants close the stomata, so as not to deplete the internal water, and active water uptake becomes more important under such non-transpiration situations. In active water uptake, one of the relevant root-related traits would be osmotic adjustment. However, using such traits is difficult in breeding programs (Turner et al. 2006).

The passive water uptake takes place by gradient of water potential from the roots to shoots, where Vapor Pressure Deficit (VPD) in the air is the principle driving force. Thus, higher VPD causes more transpiration to occur via stomata, which pulls down the leaf water potential. Subsequently, it reduces the xylem pressure potential in the stems and then in the roots. This creates a gradient in water potential, which forces the soil water into the xylem in roots and then to the leaves. Under normal circumstances, this passive water uptake plays a major role in terms of the plant water. Under the passive water uptake, the relevant root traits are root hydraulic conductivity (vertical water flow from roots to leaves) and root permeability (transverse water flow from the root surface to xylem). The root permeability could be further dissected into three different paths (1) apoplastic

(inter-cells), (2) symplastic (cell-to-cell), and (3) transcellular (cell-to-cell) (Steudle 2000). The symplastic path more closely relates to the active water uptake.

Chickpea is known to have varying root distribution across soil depths depending on the soil water availability. It has substantially smaller RLD than that of several cereals, e.g., barley (Thomas et al. 1995), but has an efficient water uptake. The difference for water uptake between chickpea and cereal species has been attributed to the function of root hydraulic conductivity, which is mainly governed by the diameter and the distribution of the meta-xylem vessels (Hamblin and Tennant 1987). Chickpea could develop its root systems upto two to three times greater in the surface soil layer (0–15 cm) at mid-pod filling stage when irrigated. On the other hand, the proportion of RLD distributed at deeper soil layers (115–120 cm) was found higher under receding soil water conditions compared to that of the well-watered condition (Ali et al. 2002). In another study, chickpea had a greater proportion of the root system in the deeper soil layer under dryland environments than field pea (Benjamin and Nielsen 2006). In addition, chickpea possesses greater root surface area to root weight ratio, compared to field pea or soybean. These studies suggest that chickpea plants are better equipped in terms of the soil water uptake to cope with the drought environments. Enhancing root traits would, therefore, be one of the promising approaches to improve drought avoidance in chickpea under terminal drought conditions.

10.4 Genetic Dissection of Root Traits

In order to target the root traits in chickpea breeding to improve drought tolerance, understanding the genetics of root traits is crucial. In the first instance, to have a knowledge about the genetic variability of root traits in chickpea germplasm, a mini core collection consisting of 211 chickpea genotypes developed by Upadhyaya and Ortiz (2001) was assessed in the cylinder culture with image capturing and analysis systems in two seasons. A large and significant variation was observed among the accessions of the mini-core collection in terms of root length density (RLD), root dry weight (RDW), rooting depth (RDp), and root to total plant weight ratio (*R/T*) (Krishnamurthy et al. 2004; Kashiwagi et al. 2005). Although a significant genotype × season interaction was observed for RLD and *R/T*, it was a noncrossover type. Therefore, a rank correlation analysis was performed between the accession means of two seasons to identify the contrasting genotypes in terms of root traits. The studies identified two accessions namely, ICC 4958 and ICC 8261, as having large and prolific root systems. In addition, the root traits of ten accessions of annual wild *Cicer* species were also evaluated in one season. The wild relatives had smaller root systems than *C. arietinum* except for the most closely related species *C. reticulatum* whose root systems were similar to that of the average root system of *C. arietinum*. It has to be mentioned here that these findings need further validation keeping in mind the effect of phenology on the timing of root growth.

Most of the wild accessions tested here were late in flowering, and these evaluations have been carried out using 35-day-old plants. As most of the wild *Cicer* species are late in phenology, it may be appropriate to measure the root system differences of wild species accessions at a later growth period.

Subsequently, in a study conducted to estimate the gene effects for root traits, two contrasting pairs of chickpea genotypes, ICC 283 and ICC 1882 (smaller roots) and ICC 8261 and ICC 4958 (larger roots), were identified for developing populations for the genetic analysis (Kashiwagi et al. 2008). In these analyses, the additive gene effect and additive × additive gene interaction have been found to play important roles in determining the RLD and RDW. In addition, the direction of the additive gene effects was consistent and toward increasing the root growth. The results encouraged the ICRISAT team to proceed with the breeding program for root systems in chickpea, although delaying selections until later generations with larger populations was proposed (Kashiwagi et al. 2008).

In order to identify the genomic regions or quantitative trait loci (QTLs) for root traits, three recombinant inbred line (RIL) populations were developed at ICRISAT. The first population consists of 257 RILs from the cross Annigeri × ICC 4958. Two other RIL populations involving parents more genetically and phenotypically distant, selected after screening the mini core collection as mentioned above, were developed: 281 RILs from the cross ICC 283 × ICC 8261 and 264 RILs from the cross ICC 4958 × ICC 1882.

The Annigeri × ICC 4958 RILs were evaluated for two seasons under terminal drought conditions, and approximately 40 molecular markers (SSR) were genotyped in the population. A QTL responsible for 33% of the phenotypic variation for root length and root biomass was detected (Chandra et al. 2004). The root trait phenotyping has been done for the two other mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261), and genotyping is underway with a variety of molecular markers. Limited level of polymorphism in intraspecific mapping populations of chickpea is a major constraint in mapping of any trait in chickpea. To aid in mapping, a set of 311 SSR markers have been developed from an SSR-enriched genomic DNA library (Varshney et al. 2007), and a set of 1,344 SSR markers have been developed after mining about 46,270 BAC-end sequences (Nayak et al. 2008). With the existing set of SSR markers in public domain and newly developed markers at ICRISAT (in collaboration with University of California, Davis, CA, USA; University of Frankfurt, Germany) and National Institute of Plant Genome Research (NIPGR), New Delhi, India (Sabhyata Bhatia, pers. commun.), more than 2,000 SSR markers are available in chickpea (Varshney et al. 2008, 2009a; Nayak et al. 2010). An integrated genetic map with 521 loci has been developed by Nayak et al. (2010). In addition to SSR markers, Diversity Arrays Technology (DArT) markers are currently being used for genotyping the two mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261). Given the large phenotypic and genotypic contrast between the parents involved in these populations and high density marker genotyping, the chances to identify additional major QTLs for root traits as defined above are high.

10.5 Transcriptomics Approaches for Identification of Genes from Root Tissues

Plant stress responses are complex and diverse, and every gene involved, from recognition to signaling to direct involvement, forms part of a coordinated response network. Controlling gene expression is one of the key regulatory mechanisms used by living cells to sustain and execute their functions. Although the final activity of a gene is determined by encoded protein, measurements of mRNA levels have proven to be a valuable molecular tool. In order to obtain a complete picture of a plant's response to stress, it would be ideal to study the expression profiles of all possible genes in its genome or at least those involved in conferring stress tolerance. Traditional approaches for undertaking genome-wide expression studies involve the use of microarray or cDNA macroarrays. Although in chickpea, transcriptomic approaches are not in an advanced stage, they progress in this direction that has already been initiated (Coram and Pang 2007).

The first step toward transcriptomics studies is the identification or cataloging of genes involved in the trait. One of the most simple and straight forward approach is the generation of expressed sequence tags (ESTs), which involves large-scale single-pass sequencing of randomly selected clones from cDNA libraries constructed from mRNA isolated at a particular developmental stage and in response to a particular stress (Sreenivasulu et al. 2002). Functional identification of sequenced clones is becoming easier by the availability of rapidly growing sequence databases, such as Genbank and genome sequence data of several crop species including the three legumes, i.e., *Medicago truncatula*, *Lotus japonicus*, and *Glycine max*.

The EST datasets can be used in gene expression/functional genomics studies to identify putative genes with differential expression and to generate the gene-based functional molecular markers such as EST-SSRs, EST-SNPs, and single feature polymorphisms (SFPs) (Varshney et al. 2005). EST analysis has become a popular method for gene discovery and mapping in cereal crops (Varshney et al. 2006). The first resource of ESTs (ca. 2800) in chickpea was developed at ICRISAT from root tissues challenged by drought stress (Buhariwalla et al. 2005; Jayashree et al. 2005). The EST library was constructed after subtractive suppressive hybridization (SSH) of root tissue from two chickpea genotypes (the landrace ICC 4958 and a popular local variety Annigeri), which were considered to possess important sources of drought tolerance (Saxena et al. 1993; Saxena 2003). A total of 2,179 ESTs were generated with putative identification that resulted into 477 unigenes. A total of 106 EST-based markers were designed from the unigene sequences with functional annotations. To enrich the resource of ESTs involved in drought and salinity stress tolerance (or response), ten different cDNA libraries were constructed from the root tissues of ICC 4958, ICC 1882, JG 11, and ICCV 2 (parental genotypes of the mapping populations segregating for drought and salinity), challenged by different types of drought (chemical induction using polyethylene glycol (PEG), sudden dehydration stress, slow drought stress to potted plants grown in the greenhouse, and prolonged drought stress under field conditions) and salinity stresses (treated

with 80 mM NaCl solution). In summary, a total of 20,162 ESTs have been generated in the study using Sanger sequencing approach at ICRISAT and have been deposited in GenBank (Varshney et al. 2009b). A detailed analysis of ESTs has provided a set of 6,404 unigenes.

In addition, "whole transcriptome sequencing" using Solexa sequencing technology (see Varshney et al. 2009c) has been initiated by ICRISAT in collaboration with colleagues from the National Center for Genome Resources, Santa Fe, New Mexico, USA (Greg May and Andrew Farmer), and the University of California, Davis, USA (Doug Cook). In this approach, the RNA isolated from drought stress challenged root tissues of different stages and were pooled for ICC 4958 and ICC 1882 genotypes separately. Half run of Solexa sequencing on the pooled RNA samples from ICC 4958 and ICC 1882 yielded 5.2×10^6 and 3.6×10^6 sequence reads (May et al. 2008), respectively. The preliminary results of the Solexa sequencing are summarized in Table 10.1. Ideally for analyzing the Solexa datasets, genome assembly (reference assembly) of the same species is prerequisite for aligning the short tags (~36 bp). In case of chickpea, however, no genome assembly was available during the analysis. To analyze the generated Solexa datasets, the following three set of sequence resources were used (1) *M. truncatula* (Mt) IMGAG (International Medicago Genome Annotation Group) gene assembly representing 29.5 Mb sequence data, (2) *C. arietinum* transcript assembly (Ca TA) of JCVI (The James Craig Ventor Institute) representing 681 kb sequence data and (3) *C. arietinum* (Ca) BAC-end sequence (Ca BES) data representing 16.4 Mb sequence data. As a result, the Solexa datasets showed matches with 5,886 and 7,338 genes in cases of ICC 4958 and ICC 1882, respectively (Table 10.1). These datasets are being analyzed for identification of gene-based SNPs between ICC 4958 and ICC 1882 so that the polymorphic genes could be integrated in the genetic maps. Such efforts should lead to the identification of drought QTL-associated genes that would be useful for molecular breeding.

Other functional genomics studies using the chickpea/legume-based gene microarrays have also been undertaken for identification of genes for drought tolerance; however, these were not exclusively focused on root traits. For example,

Table 10.1 Preliminarily gene discovery in two chickpea genotypes by employing the Solexa sequencing technology

Features	ICC 4958	ICC 1882
Number of reads	36,15,433	52,07,099
Average read length	36	36
Average read quality	26	21
Alignment with TA		
Read aligned	11,95,622 (33%)	21,22,069 (41%)
Reads uniquely aligned	5,72,751 (16%)	9,67,102 (19%)
Alignments with BES		
Aligned	10,48,614 (16%)	17,88,936 (34%)
Uniquely aligned	5,11,148 (14%)	8,54,085 (16%)
Overall number of gene matches	5,886	7,338

Boominathan et al. (2004) carried out a gene expression study of drought adaptation in chickpea using subtractive suppressive hybridization in combination with differential DNA array hybridization and northern blot analysis and identified 101 drought-inducible transcripts. Similarly, Coram and Pang (2006) developed a "Pulse Chip" microarray and applied it to identify the genes expressed in response to abiotic stresses such as drought, cold, and high salinity. In another study, transcript profiling of tolerant and susceptible chickpea genotypes under drought, cold, and high salinity was conducted (Mantri et al. 2007). These studies provide opportunities for illuminating the mechanisms of drought tolerance in chickpea and indicate the molecular pathways used by the plant as well as the function of the candidate genes involved. It would be interesting to see the colocalization of such genes with QTLs related to root trait in chickpea.

10.6 Prospects for Molecular Breeding for Root Traits

The role of root traits in conferring drought tolerance in chickpea is well established. A significant challenge to the selection for root traits is the difficulty of evaluating root phenotypes, since many root traits are phenotypically plastic, roots are difficult to extract from the soil, such extraction may change certain traits such as architecture, and many root sampling procedures are destructive. Research on drought tolerance still has to deal with many complicated aspects, especially concerning root functions. The reason is that the root is difficult to visualize and extremely sensitive to the surrounding environmental factors because of the G × E interactions. So, many efforts have been made to characterize and identify varietal differences based on root traits (Kashiwagi et al. 2005). These challenges make the prospects of marker-aided selection an attractive alternative to phenotypic selection.

The availability of appropriate molecular markers is an important prerequisite for marker-assisted selection. The availability of more than 2,000 SSR markers and DArT arrays in chickpea will enable the development of the genetic maps and mapping of traits in intraspecific populations. The integration of the candidate genes showing differential expression as well as SNPs between contrasting genotypes into QTL maps will provide genes and markers associated with root trait QTLs.

After identifying the QTLs, molecular markers associated with these QTLs need to be validated on a range of germplasm to select the most promising QTLs. For introgression of these QTLs, the drought-tolerant (possessing the QTLs) and drought-sensitive lines (showing the polymorphism at QTL with drought tolerant genotypes) are selected. After generating the F_1s by crossing the susceptible drought-sensitive varieties (recurrent) with drought-tolerant donor variety, the F_1 seeds are raised and backcrossed to the recipient varieties. After raising the BC_1F_1 population, these plants are genotyped with the identified molecular marker(s) associated with targeted QTLs. Based on marker genotyping data, the desired plants

are used further for backcrossing to produce the BC_2F_1 populations. Similar cycles of backcrossing and selection of lines with molecular markers for making them homozygous for the next generations are continued until the necessary recovery of the recurrent parent genotype is achieved. Many molecular breeding programs do not involve the use of markers in background selection. However, the availability of Diversity Array Technologies (DArTs), a low cost marker system in chickpea, creates the possibility to use DArT markers for background selection. Subsequently, the marker-assisted backcross (MABC) lines are evaluated in replications on-station and on-farm trials for agronomic performance. Eventually, the successful products of MABCs are selected and advanced to release as varieties in targeted environments.

Indeed, the above scheme of introgressing of QTLs/genes into varieties of interest has been successfully utilized in several cereal species (Varshney et al. 2006, 2007). It is anticipated that introgression of root trait QTLs in drought-sensitive chickpea varieties should be feasible in the coming years.

10.7 Looking Ahead on Root Trait Research and Applications in Chickpea

This chapter presents the importance of root traits in conferring drought tolerance in chickpea. However, molecular mechanisms of root traits at the physiological and genetic level are yet to be understood. On the one hand, the simple screening methods have been developed for precise phenotyping root traits at a large scale, enabling phenotyping of large segregating populations possible. In parallel, the genomic resources including large number of SSR markers, BAC and BIBAC libraries, BAC-end sequences, ESTs, and Solexa tags have been developed (Varshney et al. 2009a). These resources offer the possibility to develop the dense genetic map, transcript maps, and integrated genetic-physical maps of chickpea. These genomic tools should identify the root trait QTLs at a higher resolution that can be used in molecular breeding for drought tolerance in chickpea.

In order to understand the genetic basis of root traits at the molecular and cellular level, it will be possible to delimit root trait QTLs and dissect them at nucleotide level with the help of genomic resources in chickpea as well as in *M. truncatula*, *L. japonicus*, and *G. max* by using comparative genomics. The approaches like "genetical genomics" or "expression genetics" that involves the analysis of gene expression data together with the phenotyping data should provide the insights on direct involvement or regulation of QTL/gene for root trait on drought tolerance. The function of candidate genes can further be validated by using the chickpea TILLING populations recently developed at Washington State University, USA (Rajesh et al. 2007), and ICRISAT. With such available resources, we envision a more rapid understanding of the genetic and functional basis of root traits for drought tolerance.

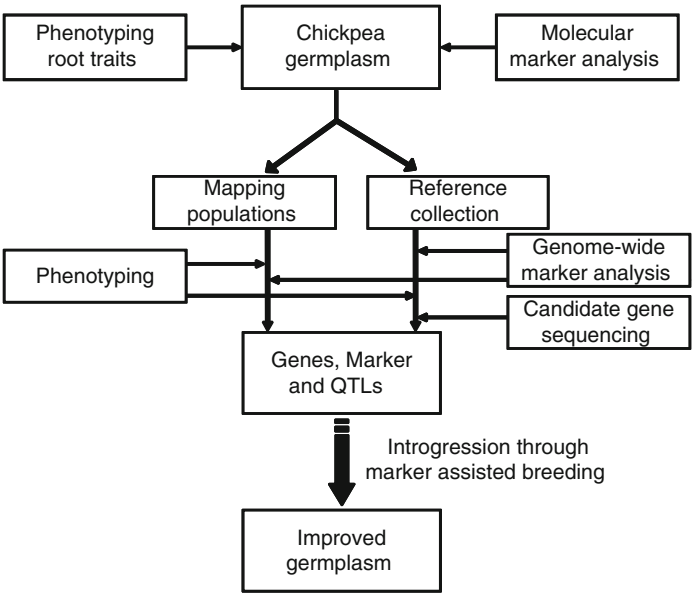

Fig 10.2 A scheme to utilize the root traits for chickpea improvement. The figure represents the holistic approach combining genomics, physiological, and breeding strategies. For instance, the molecular marker profiling and physiological screening of germplasm provides the contrasting genotypes at genetic as well as physiological level for developing (**a**) the mapping populations and (**b**) the reference collection. The mapping populations can be genotyped with molecular markers and phenotyped for root traits. Linkage analysis together with phenotyping data on the mapping population will provide the QTLs and markers associated with root traits. Similarly, the genome wide molecular genotyping or candidate gene sequencing of the reference collection together with phenotyping data for root traits can be subjected for association genetics and the markers/genes tightly associated with root traits can be identified. Molecular markers/genes identified by linkage analysis or association genetics can be used for marker-assisted breeding to introgress the drought-tolerant genomic regions from drought-tolerant genotypes into drought-sensitive genotypes to develop improved drought-tolerant cultivars of chickpea

Finally, the advancement in chickpea genomics and refinement of root physiology approaches would provide access to agronomically desirable alleles present at QTLs for root traits. A scheme has been proposed in Fig. 10.2, showing the utilization of root traits for chickpea improvement. The combined approach of genomics and physiology in chickpea breeding would enable us to improve the drought tolerance and yield of chickpea under water-limited conditions more effectively.

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