Drought Stress in Chickpea: Physiological, Breeding, and Omics Perspectives

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1 Introduction

Legumes are valuable commercial and agricultural crops: among these, chickpea is an annual crop that is generally cultivated on marginal domains with low input resources (Srinivasan 2017). It is a nutritionally rich crop having high protein contents (18–23%), hence known as 'poor man's meat.' Along with protein, it also carries some essential amino acids (leucine, lysine, threonine, isoleucine, methionine) as well as vitamins (e.g., A, K) (Jukanti et al. 2012; Sharma et al. 2013). Some legumes have antinutritional factors, such as trypsin inhibitors (soybean) and vicin (faba bean), whereas chickpea has no specific antinutritional component (William 1987). Nodules that are present on the roots of chickpea fix atmospheric nitrogen through the symbiotic relationship with soil-borne bacteria, particularly *Rhizobium*.

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Based on the symbiosis process, chickpea can achieve up to 76% of its own nitrogen requirements. It is very effective for crop rotation because it has the ability to increase soil fertility for coming crops after it is harvested (Flowers et al. 2010).

Chickpea is cultivated on 931,000 hectares in Pakistan with 359,000 tons of production annually. It occupies about 5% of the rabi-cropped area in Pakistan. India and Australia are the major producers of chickpea: Pakistan ranks third with respect to area and production. Punjab shares about 92% of the chickpea area in Pakistan for cultivation, mainly in the Thal region. The production of chickpea is very low that it is unable to fulfill the requirements of the country, so chickpea must be imported from other countries, particularly from Australia. Pakistan imported 0.450 million tons of chickpea during 2016–2017 (Govt. of Pakistan 2016–2017). Chickpea is classified into two distinct classes ('Desi' and 'Kabuli' types) based on plant type and geographic distribution. The Desi type is primarily grown in India and Pakistan and has small seeds that are brown to black in color. The Kabuli type is mostly grown in temperate regions (e.g., Ethiopia and Syria) and carries large angular seeds varying in color from cream to beige. The Kabuli type is a higher yielder than Desi, and it also has more nutritive value than the Desi type. Interestingly, the Desi type is more tolerant of drought stress as compared to the Kabuli type (Jukanti et al. 2012). Although it is a valuable crop for developing countries, it faces several biotic and biotic stresses; as a result, its production is being reduced while the area remains stagnant. Low production because of the availability of different stresses is the main fact for its cultivation.

Climate change is exerting an adverse effect on crop productivity by shifting the natural growth period. During previous centuries, an increment of 1.2 °C in temperature is recorded as caused by climate change. Moreover, it is expected that it would increase up to 3 °C by 2100 (Patwardhan et al. 2007). During high temperature, the rate of evapotranspiration is increased; consequently, reduction in soil moisture leads to the appearance of drought stress (Chaves et al. 2002). Now, this has become a global phenomenon that can affect the productivity of agricultural crops in advanced as well as developing countries. Globally, 90% of chickpea is cultivated in rain-fed areas where terminal drought stress is the main limiting factor for its growth and production (Srinivasan 2017). Drought stress is the second most important growth-restraining factor after diseases in chickpea (Mohammadi et al. 2011). Transient and terminal drought stress are the most common types of drought, based on the duration of effect. In the short term, shortage of water can affect the plant at any stage of its development and can be remedied by precipitation, whereas terminal drought stress is a long-term stress that creates a constant water deficit condition which hinders the reproductive stage of crop plants. Semi-arid tropics and Mediterranean climates are mostly affected by terminal drought stress (Li et al. 2018).

Drought stress has a severe effect on flowering and seed formation. Throughout the world, terminal drought stress is reducing chickpea yield as much as 40–50% annually (Kumar and Abbo 2001; Ramamoorthy et al. 2017). Oxidative stress is produced by reactive oxygen species (ROS) such as H_2O_2, O_2^-, O^-, and HO^-, which result in the production of a toxic environment for plants. Oxidative stress

deteriorates the normal behavior of different metabolic pathways in the cell. The activity of antioxidants such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), peroxidases, glutathione peroxidase (GPX), and ascorbate peroxidase (APX) is increased or decreased under drought stress depending upon the tolerance or susceptibility of the plant (Mohammadi et al. 2011). Proline is accumulated in tolerant plants under drought stress to reduce the effect of stress (Dalvi et al. 2018).

In this scenario, the use of omics-based breeding strategies along with conventional techniques is indispensable. In the present era, the availability of next-generation sequencing (NGS) tools provides a comprehensive platform to maximize the use of these omics approaches. The genome of Desi type (ICC 4958) and Kabuli type (CDC Frontier) have been completely sequenced through NGS and their genome assemblies are publicly available (Jain et al. 2013; Varshney et al. 2013b). The sequenced genome was used to develop a genome-wide physical map of chickpea (Varshney et al. 2014a).

Now omics approaches, such as genomics, transcriptomics, proteomics, metabolomics, ionomics, and phenomics, as well as genomic-assisted breeding (marker-assisted recurrent selection and marker-assisted backcrossing), have been applied in chickpea to accelerate the breeding program (Varshney et al. 2014a). RNA-Seq-based differential gene expression is used for the identification of novel genes and associated pathways under different environmental platforms. The availability of diverse germplasm and NGS tools will help the plant breeder to design an appropriated breeding plan to combat drought stress.

2 Impact of Drought on Chickpea

Chickpea is vulnerable to drought stress, so we must face its drastic effect. The effect of drought stress is mainly associated with the stage of the crop as well as the duration of the stressed conditions. All the impacts created by drought stress ultimately result in the reduction of yield and quality as well. Here, some of the prominent effects of drought stress are discussed.

2.1 Effect at Vegetative and Reproductive Phase

There are two main early drastic effects of drought stress, associated with the seedling stage as well as deprived seedling stand in plants (Harris et al. 2002). The effect of drought stress at the time of the vegetative phase is less as compared to the anthesis phase (Mafakheri et al. 2010). The drought-tolerant chickpea variety (Bivaniej) gave more yield as compared to the susceptible variety (Pirouz). The loss of yield was also associated with the phase of stress imposition, either vegetative or anthesis. Application of terminal drought stress, at the time of early stages of pod formation,

was responsible for lower yield by reducing the reproductive growth, biomass, seed yield, and harvest index in chickpea (Pang et al. 2016). Flower abortion and empty pod formation had a substantial effect on yield reduction under the terminal drought environment. Pollen viability was decreased under lower soil moisture level. Historically, it was recorded that the yield and productivity of chickpea remained low under severe dehydration conditions. The normal pollen growth is reduced under drought stress, and consequently the number of sterile pods is increased. Ultimately, the yield is reduced by the increased number of empty pods and reduced seed size under drought stress.

Similarly, the process of seed-filling is disturbed under drought conditions, leading to the development of chickpea seeds of a small size (Kalra et al. 2008). Based on a comparative study of Desi and Kabuli types of chickpea, it was observed that the Desi type had more tolerance against drought stress (Muruiki et al. 2018) because it has a better genetic makeup that is intrinsically inherited. The nodulation process is badly affected by drought stress in chickpea. The number, size, and vigor of nodules are reduced in the presence of drought stress, resulting in inferior nitrogen fixation (Muruiki et al. 2018). The yield potential of chickpea is decreased by abnormal nodule development. Flowering, pod formation, and seed-set are three more sensitive stages in chickpea during drought stress. Roots are the primary outgrowth in plants, responsible for the absorption of water and nutrients from the soil. Roots have a significant effect on the efficiency of transpiration pull, used to extract the water from the soil. If the plant has a deep root system, then it will be good absorber of water, present either at the upper surface or underground in the soil. On the other hand, shallow and dense roots might be good providers of nutrient uptake such as phosphorus, found at the upper surface of the soil (Ramamoorthy et al. 2017).

The reproductive phase is the most critical stage under drought stress, particularly associated with yield potential. At the time of pod-filling, if drought stress occurs, then flower-shedding and pod-abortion will occur, and thus the yield will be reduced by producing fewer seeds with lower seed weight (Pang et al. 2016). Yield losses from drought stress ranged from 40% to 50% in chickpea (Muruiki et al. 2018). The yield potential of chickpea under drought stress was found to be strongly correlated with leaf osmotic potential, leaf water potential, and relative water content (RWC) (Summy et al. 2016). Drought stress had a drastic effect on these parameters, particularly in the susceptible genotypes of chickpea; consequently, the seed yield fell as much as 37.32% in the susceptible genotype, HC-1. The major constraint for the production of chickpea is terminal drought stress, which affects mainly the reproductive stage of the plants. In chickpea, the range of reduction in flower formation, pod formation, and yield was 37–56%, 54–73%, and 15–33%, respectively, under drought stress (Fang et al. 2009).

Moreover, the effect of drought stress on germination and pollen viability was also evaluated by in vitro assessment (Rokhzadi 2014). It was found that in the chickpea genotype (Rupali), the decrease in germination and pollen viability under drought stress was 50% and 89%, respectively. In comparison to in vitro, the rate of pollen reduction was higher, 80%, when exposed to drought stress in vivo. Based on plant

growth regulators, it was also reported that the drought effect was more pronounced during the reproductive stage as compared to vegetative. These results were used to further justify the drastic effect of terminal drought stress in chickpea. So, by seeing the visual effects of drought stress on crop plants, it can be stated that drought stress is the growth-limiting factor, resulting in inferior vegetative and reproductive growth of the plant.

2.2 Effect on Photosynthesis

Photosynthetic machinery is the primary index to observe the health, vigor, and potential of a plant to rescue itself under water deficit conditions. The rate of photosynthesis is reduced under drought stress because of the disturbance of several metabolic pathways. Reduction in photosynthesis causes a reduction in the yield by altering some physiological mechanisms. Thylakoid membrane and chlorophyll pigments are the basic and vital parts of the photosynthetic apparatus. Both these organelles become less efficient under drought stress, resulting in leaf necrosis and reduction in photosynthesis efficiency. Leaf area is also associated with photosynthesis, so reduction in leaf area will result in a lower level of photosynthetic process in the presence of drought stress. Because the production of glucose is decreased, so that a lower amount is available for the plant to use, the production of new leaves is reduced. On the other hand, the rate of leaf abscission is increased, to save the photosynthetic product (glucose) for plant survival (Tas and Tas 2007).

In tolerant chickpea genotypes, the photosynthetic regulatory genes transcribe into β-carbonic anhydrase-5 under drought stress (Das et al. 2016). The interaction of CO_2 with RuBisCO is immersed by a specific combination of RuBisCO and carbonic anhydrase. This combination is used for the effective mechanism of RuBisCO to perform the proper carboxylation process. Carbonic anhydrase can be used as a marker for the selection of drought-tolerant chickpea genotypes in screening experiments. Similarly, at the time of vegetative and anthesis phase, decrease in chlorophyll content, that is, chlorophyll *a*, chlorophyll *b*, and total chlorophyll content was observed under drought stress in chickpea (Mafakheri et al. 2010). The rate of photosynthesis is primarily determined by the resistance of mesophyll under drought stress (Rahbarian et al. 2011). Based on mesophyll tolerance under drought stress, it was shown that the rate of photosynthesis was higher in the drought-tolerant chickpea variety (Bivaniej) as compared to the susceptible one (Pirouz). Similarly, a significant reduction in chlorophyll content, chlorophyll fluorescence, photosynthesis, and PSII-photochemical efficiency (Fv/Fm) was observed in chickpea genotypes under drought conditions at the seedling stage.

Another study also showed that positive osmoregulation and leaf turgor had a significant association with the photosynthetic machinery (e.g., photochemical efficiency of PS-II) under drought stress (Basu et al. 2007b). Leaf turgor and osmoregulation also maintain photosynthetic efficiency by securing normal activity of PS-II under drought stress in chickpea. The effect of drought stress on photosynthesis can

be measured through chlorophyll *a* fluorescence (Kalefetoğlu Macar and Ekmekçi 2009). Increments in the duration of drought stress were responsible for the photoinhibition of PS-II activities in chickpea. There are certain reasons for the reduction of photosynthesis under drought stress; among these, the activation of sucrose-phosphate synthase (SPS) and production of hexose sugars (carbohydrates) are of prime importance (Basu et al. 2007a). When leaf starch starts to decline, carbohydrate and SPS increase, and both these factors are responsible for the accumulation of sucrose. It was experimentally approved that drought-induced alterations were associated with SPS and carbohydrates, which modify the efficiency of water uptake in leaves. So, the rate of osmotic adjustment, photosynthesis, and RWC under drought stress is primarily associated with the SPS and carbohydrate accumulation under drought stress.

2.3 Effect on Water Relationships

Drought stress can be measured by determining water status in the plants, called relative water content (RWC). The lower level of moisture content in plants leads to the reduction in available RWC present in the plants. The genetic makeup of the plants also has a prominent effect on the plants for maintaining their level of RWC under varying levels of moisture. The variation in RWC is produced by the plant ability to obtain water from the soil through the roots. RWC is retained by creating a high water potential gradient, reducing water losses by controlling the stomatal openings, and increasing root length (Omae et al. 2005). RWC is proposed as the best choice for the representation of current water status in terms of genetic variation, based on the genetic association between RWC and production during drought stress. In chickpea, about 85% increment in RWC was recorded in the tolerant chickpea variety (JG-62) as compared with other susceptible varieties under drought stress (Bhushan et al. 2007). The increment in RWC is linked by the accumulation of proline content as well.

The movement and retention of water are controlled by the stomata in plants under stress. Stomatal indices were varied among leaves of well-watered and drought stress environments. Stomatal indices under drought stress were lower in the leaves and vice versa under well-watered leaves (Hamanishi et al. 2012). Drought stress has a significant effect on the opening and closing of stomata (Buchanan et al. 2005). Under drought stress, most often stomatal closure is increased as a result of the biosynthesis of abscisic acid (ABA), the stress hormone, which is also increased. The stomatal opening is closed by stomatal guard cells with the help of ABA, making the leaves turgid. The rate of photosynthesis, as well as water usage, are decreased under drought stress. Ultimately, the normal growth rate of the plant is disturbed by chlorophyll necrosis. Because water is a deficit under drought stress, to overcome the effect of that stress the plant uses water efficiently, that is, water use efficiency. Drought-tolerant chickpea genotypes have high water

use efficiency as compared to susceptible types (Rahbarian et al. 2011). Normally, water use efficiency is increased under drought stress, especially in tolerant plants. The same process was seen in chickpea, in that the water use efficiency was higher in tolerant genotypes (MCC-877 and MCC-392) compared with susceptible genotypes (MCC-448 and MCC-68).

Further, water use efficiency seemed to be increased significantly from seedling stage to early flowering and was reduced quickly during pod filling under drought stress (Basu et al. 2007a). RWC was decreased significantly under drought stress among susceptible genotypes that were unable to counter the effect of drought stress accurately during early growth stages. Terminal drought stress was responsible for the reduction of leaf water potential, that is, −1.00 MPa to −2.25 MPa from prestress level to terminal drought stress level in chickpea. Gradual changes in RWC were observed under certain levels of drought stress; consequently, the osmotic adjustment values were changed significantly in many chickpea genotypes. Drought stress is a reason to limit the RWC in plants by reducing the moisture level in the soil (Zaman-Allah et al. 2011a), whereas the tolerant chickpea genotype has developed the process through which these plants can conserve or save water when a plant needs no more water for its growth and development. It was assumed that the previously saved water will be available for later reproductive stages, that is, flowering and podding in chickpea under drought stress. In contrast to tolerant chickpea genotypes, susceptible genotypes were more prone to drought stress because these were users of more water during early vegetative growth stages. Similar findings were recorded in chickpea for water uptake profile against drought stress (Zaman-Allah et al. 2011b). Tolerant and susceptible genotypes had a clear and distinct type of water profile for drought stress. Root traits, that is, root depth and density, had no clear and distinct criteria among tolerant and susceptible genotypes at the time of the reproductive phase. The main fact about tolerance genotypes was that they conserve water during the vegetative stage equally from a stressed and control environment. That conserved water was used to reduce the canopy conductance; thus, the favor was given to the reproductive stage with a successful completion of the life cycle. Therefore, the temporary pattern of water uptake as adopted by the plant roots is more valuable for the development of drought tolerance as compared to root growth. This process can be used to understand the plant behavior, that is, how the plant can maintain its RWC under terminal drought stress.

2.4 Effects at Molecular and Cellular Level

Several stress-responsive genes, such as ABA-regulatory genes and transcription factors, were identified in some model plants as well as in crop plants (Zhu 2000). Stress tolerance is increased through the regulation of drought-responsive genes, either by direct upregulation of the target genes or by regulating the transcription factors of these stress-responsive genes (Haake et al. 2002).

Cell division has a key role in plant growth and developmental processes. Under drought stress, the normal functioning of cell division is reduced (Taiz and Zeiger 2006). Subsequently, cell membrane stability is reduced, which leads to the reduction of cell growth, and finally, growth is reduced. The movement of water from xylem to extended cells is interrupted under severe water deficit conditions. Reactive oxygen species (ROS), such as H_2O_2 (hydrogen peroxide), O_2^- (superoxide), O^- (singlet oxygen), and HO^- (hydroxyl radicals) are produced in plants under a stressed environment (Rahimizadeh et al. 2007). These ROS are highly toxic to plants and can reduce the quality as well as production potential of crops. ROS produce oxidative stress, damage the normal plant metabolism by altering the cellular changes in membranes, nucleic acids, and proteins. ROS disturb the normal metabolism of the cell through protein denaturation, nucleic acid mutation, and lipid peroxidation (Joseph et al. 2011).

Drought stress has a significant effect on the normal functioning of plants, as cell membrane stability, osmotic regulation, RWC, seedling growth rate, and inhibition retention are reduced under drought stress. Tolerant chickpea cultivars (RSG-143-1, RSG-44, ICC-4958) were found to show lesser effects of drought stress as compared to susceptible cultivars (Pant-G-114) that were unable to avoid the drastic effects of drought (Gupta et al. 2000). Electrolyte leakage under drought stress was altered along with other traits of drought significance. Stomatal conductance, the efficiency of PS-II, and RWC are the main factors that were associated with the tolerance of chickpea plants under drought stress (Pouresmael et al. 2013). Thus, these traits should be characterized ahead of several other factors in deciding selection criteria for the identification of drought-tolerant chickpea plants. ROS affect the electron transport chain, chlorophyll content, PS-II protein (D_1), and some molecules of high energy, for example, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) (Pagter et al. 2005). When the production of ROS is higher than the antioxidant defense system, cellular function is damaged (Almeselmani et al. 2006).

Chloroplasts damaged by overproduction of ROS ruined the protein–pigment complex as well as the thylakoid membrane (Farooq et al. 2017). The presence of these ROS in excess can cause cell death by denaturing the lipids, proteins, and DNA contents. The defense mechanisms of plants are activated under drought stress in response to ROS. These ROS are considered as a secondary messenger for the activation of the defense system in plants. The capability of roots to absorb water is reduced under drought stress. Similarly, translocation of sap in the phloem tissues is also reduced; consequently, a substantial reduction in plant morphological features such as antioxidant activity and nutrient uptake occurs (Armand et al. 2016). The higher accumulation of H_2O_2 and MDA content in the cell under drought stress is an indicator for drought susceptibility (Kaur et al. 2013). Susceptible chickpea genotypes were found with a higher amount of H_2O_2 and MDA content during drought stress. The tolerant genotype exhibited a higher accumulation of SOD and CAT in the cells to reduce the effect of drought stress.

3 Breeding Strategies for Drought Tolerance

Breeding strategies are used for the identification and development of tolerant varieties. The integration between conventional and nonconventional (omics-based) breeding techniques is the ideal way to accelerate the conventional breeding system for drought stress. The role of these techniques is discussed next.

3.1 Conventional and Mutation Breeding

Conventional breeding has been used in plant improvement, especially for yield and resistance to biotic and abiotic stress. As used historically in agriculture by plant breeders, it includes various methods such as introduction, hybridization, and selection for the improvement of plant architecture. The process of introducing genotypes/plants/groups of genotypes into a new environment, where these were not previously being grown, is known as an introduction. Superior varieties are imported from other countries for the improvement in the varietal developmental program. Selection becomes more effective among diverse germplasms. There are two ways to release variety through the introduction: primary and secondary introduction. If the introduced genotype is released directly for the general cultivation without any changing, this is recognized as primary introduction, for example, semi-dwarf rice and wheat varieties. In contrast, the release of introducing variety for general cultivation after making some modification is based on either selection or hybridization with local varieties is known as secondary selection (Allard 1960). The sharing of germplasm across the world is a way to increase genetic diversity and enhance collaboration among the scientific community. Genetic material is exchanged between different international research institutes, including the International Crops Research Institute for the Semi-Arid Tropic (ICRISAT) and the International Center for Agricultural Research in the Dry Areas (ICARDA) to increase genetic diversity in chickpea germplasm. ICRISAT and ICARDA are the major institutes for the chickpea germplasm collection (Table 1). The center of diversity (e.g., Turkey and Syria for chickpea) has a key role in the adaptation of plants in a changing environment.

After introduction, the other breeding method is hybridization, used to combine desirable genes found among two or more parents. Selection of better parents for novel traits in plant breeding is the first step for the genetic improvement and architecture for crop plants. After selection, it is the prerequisite to make a better combination of these traits, to fix the genetic variation. Hybridization is the basic technique, which is used very commonly in plant breeding to attain the desired combination of genes. Desired traits are transferred into the hybrid progeny and subjected to evaluation for better performance by comparing with their parents. Chickpea is a self-pollinating crop, so the rate of natural cross-pollination is very low. Artificial pollination in the chickpea is difficult because it carries small floral parts that are

Table 1 Chickpea seed banks

Sr. No.	Seed bank	Web link	No. of chickpea accessions
1	International Center for Agricultural Research in the Dry Areas (ICARDA)	www.icarda.org/	13,065
2	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	https://www.icrisat.org/	18,963
3	United States Department of Agriculture (USDA)	https://www.usda.gov/	6107
4	Plant Genetic Resources, National Agriculture Research Center, Islamabad (NARC)	www.parc.gov.pk/	2243
5	National Bureau of Plant Genetic Resources India	www.nbpgr.ernet.in/	15,986
6	Seed and Plant Improvement Institute Iran	www.spii.ir/HomePage.aspx?lang=en-US	5600
7	Aegean Agricultural Research Institute Turkey	https://arastirma.tarimorman.gov.tr/etae/Sayfalar/EN/Anasayfa.aspx	2063
8	Biodiversity Conservation and Research Institute (Ethiopia)	www.ebi.gov.et/	1156
9	Uzbek Research Institute of Plant Industry (Uzbekistan)	https://www.genesys-pgr.org	726
10	Bangladesh Agricultural Research Institute (BARI)	http://www.bari.gov.bd/	666
11	Plant Gene Resources of Canada (PGRC)	pgrc3.agr.gc.ca/index_e.html	641
12	Institute of Crop Germplasm Resources, CAAS, Beijing, China	http://www.cgris.net/default.asp	567
13	Agricultural Botany Division (Nepal)	https://www.gfar.net/organizations/agriculture-botany-division-nepal-agricultural-research-council	424
14	National Institute for Agronomic Research (INRA) (Morocco)	www.ias.csic.es/medileg/inram.html	332
15	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)-Atersleben (Germany)	https://www.ipk-gatersleben.de/en/	310

also more delicate and sensitive as compared to other crops. Handling these flowers is not an easy task: only a 10–50% success rate of artificial cross-pollination is reported in chickpea (Salimath et al. 2007).

Crop wild relatives are the imaginary source of genetic variation. These relatives are found in the different surroundings around the world, mostly in the threatened areas of degradation. The conservation of genetic variation through ex situ or in situ means is very important for the security of wild relatives. To overcome the status of

limited variability, wild relatives were exploited in a breeding program for the development of new varieties with better yield, adaptation, and resistance. Wild relatives are considered as a reservoir of variation for crop plants, that is, a potential source of adaptation that has been declining gradually in the domestic germplasm. For the improvement of genetic variability, intra specific and wide hybridization techniques have been used in cultivated chickpea. The genus *Cicer* carries nine annual and 34 wild perennial species. Among these annual species, only *Cicer arietinum* has been cultivated until now. Information about the genetic relationship of cultivated and wild species is the prerequisite, to study the evolutionary process of cultivated species as well as wild species. Wild species can be used potentially by understanding the crossing compatibilities, cytogenetic affinities, and chemotaxonomic associations between wild and cultivated plants. Methods such as interspecific hybridization, isozymes, molecular markers, and karyotypes have been used for the investigation of the relationship between wild and cultivated species of the genus *Cicer* (Hawkes 1977).

Resistant genes are mostly present in wild species, against biotic and abiotic stresses. The introgression of these resistant genes into the domestic chickpea through breeding is used for the development of tolerant varieties (Hufford et al. 2013). Therefore, the concept of 'crop re-synthesis' was widely used to recover and develop actual resistance in the plant. The development of effective hybrids by using wild relatives also has some restrictions in chickpea. Hybrid breakdown and sterility accompany a diverse group of *Cicer echinospermum* (Kahraman et al. 2017). Differential genetic loci among wild relatives are the source of sterility. The cross between cultivated and wild relatives (*Cicer echinospermum*) was studied in chickpea. The genetic diversity of chickpea can be enhanced via distinct and wide hybridization.

A comparison was made for drought stress between cultivated (*Cicer reticulatum*, *Cicer pinnatifidum*, *Cicer echinospermum*) and wild (*Cicer songaricum*, *Cicer oxydon*, *Cicer anatolicum*, *Cicer montbretii*, *Cicer microphyllum*) chickpea plants (Toker et al. 2007). Perennial species were more tolerant to drought stress than annual and cultivated species of chickpea. *Cicer anatolicum* was used to create drought resistance in cultivated chickpea because it has high affinities for compatibility with cultivated chickpea for the breeding program. Distant hybridization between cultivated and wild chickpea (*Cicer reticulatum*) was effectively exploited for the introgression of genes associated with drought tolerance (Hajjar and Hodgkin 2007).

On the other hand, mutations are the prominent source of de novo variation, particularly in self-pollinated crops. By using mutation breeding, the genetic makeup of chickpea can be diversified with the objective of increasing yield and resistance to biotic as well as abiotic stress. The mutation is used to generate desired treats in crop plants, by using chemical or physical mutagens. Chickpea cultivars were also released as a commercial variety, developed through mutation breeding (Salimath et al. 2007). Mutation breeding is recognized as a beneficial technique for broadening genetic variability and adaptability in self-pollinated as well as cross-pollinated crops.

The reverse genetic approach has a significant role in mutation breeding. In this technique, the development of a nearly isogenic line and mutants is the potential source for functional genomics (Ali et al. 2016). On the basis of evaluation through

induced mutation, *Cicer reticulatum* is documented as a drought-tolerant chickpea accession (Toker et al. 2007; Toker 2009). The Nuclear Institute for Agriculture and Biology (NIAB) is working on chickpea mutation breeding in Pakistan. Desi and Kabuli chickpea varieties of NIAB, developed through mutation, have resistance to biotic and abiotic stresses with high yield. A significant increment in the production, as well as tolerance against biotic and abiotic stresses, were reported (Haq 2009). According to the report of the International Atomic Energy Agency (IAEA), CM-72, -88, -98, and -2000 were developed by using physical mutagens at the rate of 150 Gy, 100 Gy, 300 Gy, and 150 Gy γ-rays, respectively (Table 2). On the other hand, CM-2008 was developed by using a chemical mutagen, 0.2% EMS (ethyl methanesulfonate). These varieties were high yielding and resistant to diseases such as blight and wilt in chickpea (Maluszynski 2001; Lestari 2016). The use of these varieties in a breeding program is associated with the genetic variability of chickpea against stresses. Conventional breeding methods are the basic ways for plant breeding, but omics-based breeding can be used as a supplement to increase the efficiency and worth of conventional breeding by reducing time and targeting exactly the desired genes against drought stress. Thus, the integration between these breeding methods is the key to develop drought-tolerant chickpea accessions.

Table 2 Mutant varieties of chickpea

Sr. no.	Variety name	Year	Country	Improved characters
1	Hyprosola	1981	Bangladesh	Early maturing, higher yielder, more biomass
2	CM-72	1983	Pakistan	Blight resistant and high yielding
3	Kiran	1984	India	Early maturing and salt tolerance
4	Pusa-408	1985	India	Blight resistance, high yield
5	India	1985	India	Wilt resistant and >2 seeds/pod
6	Pusa-417	1985	India	Wilt and pod borer resistant
7	NIFA-88	1990	Pakistan	Earlier maturing, high yield (15–20%) and N_2 fixation
8	Line-3	1992	Egypt	High yielding with profuse branches
9	CM-88	1994	Pakistan	*Ascochyta* and *Fusarium* resistance with high yield
10	NIFA-95	1995	Pakistan	Bacterial blight resistance
11	CM-98	1998	Pakistan	*Ascochyta* and *Fusarium* resistance
12	CM-2000	2000	Pakistan	High yield and resistance to diseases
13	Hassan-2K	2000	Pakistan	High yielding, bacterial blight resistance
14	Binasola-3	2001	Bangladesh	Early maturity
15	BGM-547	2005	India	Bold seed size
16	Pusa-547	2006	India	High yielding, *Ascochyta* and *Fusarium* resistance
17	TAEK-SEGAL	2006	Turkey	High yielding and *Ascochyta* resistance
18	CM-2008	2008	Pakistan	Bold seed and *Fusarium* resistance
19	THAL-2008	2008	Pakistan	*Fusarium* resistance, large seed size
20	Binasola-5	2009	Bangladesh	Early maturing and high yielding
21	Binasola-7	2013	Bangladesh	Tall, greater 100-seed weight
22	Binasola-9	2016	Bangladesh	High yielding and suitable for late sowing
23	Binasola-10	2016	Bangladesh	High yielding and early maturing

3.2 Omics Approaches

Omics approaches are collectively intended for the quantification and characterization of biological molecules, which are translated for various purposes, such as structure, dynamics, and function of different organisms. Different types of omics—genomics, transcriptomics, proteomics, metabolomics, ionomics, and phonemics—have been widely used for the characterization of different responses in plants, under varying environmental conditions. Large-scale genomic resources are produced with the invention of NGS and genotyping technologies such as genomics, transcriptomics, proteomics, and BAC-end sequences (BESs) in chickpea (Varshney et al. 2013a). "Omics" is one of the most imperative fields of science, standardized in recent years. This approach facilitates the identification of novelty genes, proteins, and metabolites against any stress, including drought stress. Functional characterization of the desired genes is also done by using omics approaches (Zargar et al. 2011). Similarly, omics predict the assignments of the genes, proteins, and metabolites, and assess the alterations in plants produced by different environmental conditions (Baginsky et al. 2010). Advances in genomics increased after the postgenomic era as semi-quantitative RT-PCR, real-time polymerase chain reaction (PCR), massively parallel signature sequencing (MPSS), serial analysis of gene expression (SAGE), microarray technologies, and currently NGS-based genome-wide transcriptome analysis via RNA-Seq have become more prominent and comprehensive for the identification of stress-responsive genes in plants (Singh et al. 2015). Although omics-based breeding is a comprehensive and efficient approach for plant breeding, the integration of omics-based breeding strategies with conventional breeding is seen to be effective. These techniques work parallel to each other for the improvement of drought tolerance in chickpea (Fig. 1).

3.2.1 Genomic Resources

Genomic resources have become an effective source for omics studies because of the availability of efficient genomic tools in the recent era. The genome size of chickpea is comparatively small among other legumes, such as faba bean, soybean and lentil. Thus, small genome size, as well as accessibility to NGS, offers a platform for the development of chickpea genomic resources. Plant genomics resources are emerging by the gradual development of scientific technologies in recent years, resulting in the creation of genomic resources publicly for major crops as well as for minor crops. A range of genomic resources can be retrieved through various public databases, such as the National Center for Biotechnology Information (NCBI), having indices of ESTs of different plant species for abiotic stress tolerance. The NCBI database has information vis-à-vis genetic maps, DNA markers, and transcriptome assemblies, available for various crops publicly, as in chickpea. Similarly, SNPs databases as well as some other important genomic resources were developed and notably used to enhance the working efficiency of the breeding program for

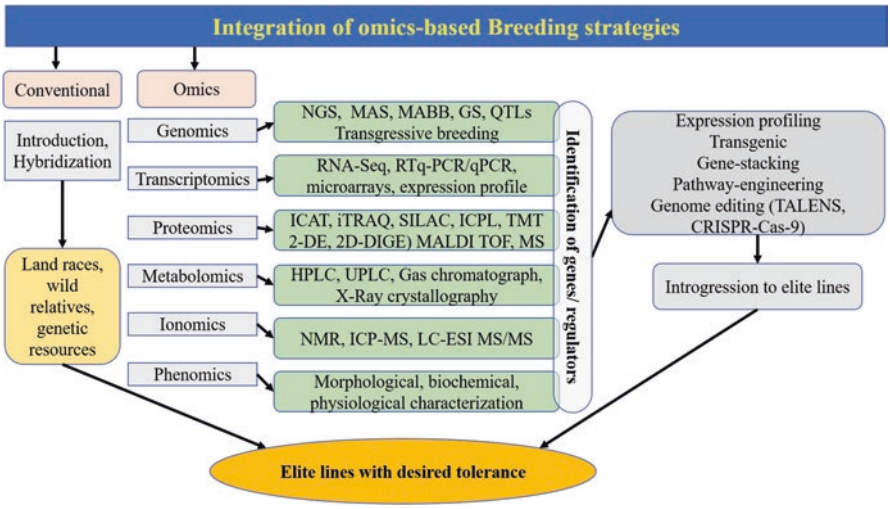

Fig. 1 Integration of omics-based breeding strategies for the development of drought tolerance in chickpea

crop improvements (Table 3). Different genetic applications such as marker-assisted backcrossing, marker-assisted recurrent selection, genomic selection, quantitative trait loci (QTL) mapping, and gene/QTL pyramiding have been massively explored and have an association with genomic techniques. Nevertheless, DNA markers are the more pertinent techniques, used in plant breeding practices, for germplasm characterization, seed purity determination, phylogenetic analysis, F_1 evaluation, and particularly for marker-assisted breeding programs (Collard and Mackill 2007). The reference genomes of chickpea varieties Desi and Kabuli were used for the improvement in assemblage and annotation of the chickpea genome (Gupta et al. 2016; Parween et al. 2015). The whole genome-wide analysis is more effective and easier, as supported by high-throughput whole-genome resequencing technologies. Polymorphisms and InDels were identified in the chickpea genome for drought stress. On the basis of whole-genome resequencing technology, different genomic regions related to nitrogen fixation and yield under drought and heat stress in the field were identified in chickpea (Sadras et al. 2016). Moreover, quick progress was made in the ultradense genetic map: whole genome-wide resequencing, genotyping by sequencing for the creation of novel single nucleotide polymorphisms (SNPs), whole-genome resequencing, mapping, and identification of potential candidate regions on the genome through NGS-based bulk segregation analysis, genome-wide association studies, and epi-genomic properties were attained (Jha 2018; Garg et al. 2016). The genomic region related to drought stress (QTLs) has significant information for the improvement of chickpea genotypes. Several QTLs associated with drought stress tolerance have been identified in chickpea (Table 4).

In this scenario, different studies have been conducted for the identification of QTLs in chickpea. The very first identification of QTLs-linked markers under

Table 3 Chickpea databases

Sr. No.	Database	Web link	Description
1	Chickpea Transcriptome Database (CTDB)	https://www.nipgr.res.in/ctdb.html	Provides information for chickpea transcriptome
2	Legume IP	http://plantgrn.noble.org/LegumeIP	Currently hosts large-scale genomics and transcriptomics data of legumes
3	National center for Biotechnology Information (NCBI)	https://www.ncbi.nlm.nih.gov/search/all/?term=Chickpea	Associated with several databases with biotechnology and biomedicine and major source for bioinformatics tools and services
4	Legume Information System (LIS)	https://legumeinfo.org/organism/Cicer/arietinum_CDCFrontier	Provides access to genetic and genomic information for major legumes
5	Kyoto Encyclopedia of Genes and Genomes (KEGG)	https://www.genome.jp/kegg/	High-level functions, such as the cell, the organism, and the ecosystem, from molecular-level information
6	Cool Season Food Legume Crop Data Base Resources	https://www.coolseasonfoodlegume.org/organism/Cicer/arietinum	Carries three versions of chickpea genome
7	Chickpea Micro-Satellite Database (CicArMiSatDB)	https://cegresources.icrisat.org/CicArMiSatDB/	Have information of SSR from the sequenced genome
8	Chickpea SNP In-Del Data Base (CicArVarDB)	https://cegresources.icrisat.org/cicarvardb/	Deals with variations around specific regions linked with QTLs
9	Chickpea Root Expressed Sequence Tag Database	https://www.icrisat.org//?s=Chickpea+Root+Expressed+Sequence+Tag+Database	Contain information about ICRISAT's root EST database
10	CicerTransDB	http://www.cicertransdb.esy.es/	Concerned with motif architecture, domain, and gene ontology

Table 4 Summary of quantitative trait loci (QTLs) linked with drought stress tolerance in chickpea

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
SSR markers				
Grain yield	1, 4	H5A08-TA8, H1B17-TA72	–	Rehman et al. (2011)
Days to flowering	1, 3, 4, 8, 8	H5A08-TA8, TA6-NCPGR12, TA132-GA137, TA159-GA6	15, 22, 5, 8	
Days to maturity	1, 3, 7	H5A08-TA8, TA6-NCPGR12, TA28-CaSTMS25	13, 22, 5–6	
Reproductive period	1, 3, 7	H5A08-TA8, TA6-NCPGR12, A28-CaSTMS25	6, 6, 5–6	
Plant height	1, 4	H5A08-TA8, H1B17-TA2	24, 7	
Harvest index	1, 3	H5A08-TA8, TA6-NCPGR12	13	
Drought tolerance score	1, 3, 7, 8	H5A08-TA8, TA6-NCPGR12, TA28-CaSTMS25, TS12-TA118	13	
Stomatal conductance	7, 7, 3	TA28-CaSTMS25, TA180-H1A10 (290bp), TA125-NCPGR10	7, 8, 9	
Canopy temperature differential	1, 3, 4, 6, 6, 6	H5A08-TA8, TA6-TS58, GA137-TA46, TR7-TA14, TA80-GA21, TA80-GA21, GA21-CaSTMS2	15, 7, 13, 8–13, 8–13, 8–13	
Drought resistance score	3, U, 3, 3, 3, 4, 3	H6C-07, H4D-11, NCPGR-81, H1B-04, H6C-07, H5G-01, H6C-07	23.3, 10.9, 6.4, 6.7, 12.5, 5.1, 15.2	Hamwieh et al. (2013)
100-seed weight	3, 3, 7, 5, 3, 4, 3	NCPGR-50, TR-50s, SCEA19, TAA-58, TS-43, H1H-15, TA-11	17.2, 6.4, 7.1, 5.9, 7.4, 11.8, 7.3	
Pod number	3, 7, 3, 3, U, 4, 3	H6C-07, TAA-55, H3G-09, H1B-04, TS-19, H5G-01, H6C-07	22.7, 9.6, 7.4, 7.3, 5.1, 6.1, 5.3	
Days to flowering	3, 4, 3, 4, U, 3, U, U, 3	H1F-14, H5G-01, H6C-07, H1B-17, TS-19, H4G-07, TS-19, GA-16, H6C-07	5.1, 5.3, 17.7, 7.8, 6.6, 9.7, 6.5, 5.3, 24.2	
Grain yield	3, U, 4, 3, 3, 3	H6C-07, H5E-02, H5G-01, H6C-07, H1B-04, TR-31	12.4, 6.7, 6, 5.3, 5.4, 6.4	
Plant height	3, 2, U, U, 2, U, 7, U, 1, U	TA-179, TA-103, TA-76, TA-76, TA-96, TA-96, TA-76, TAA-55, TA-76, TA-203, TA-113	8.8, 5.9, 5.3, 6.1, 8.5, 5.1, 5.1, 4.8, 7.3, 7.3	
Days to maturity	1, 3, 3, 3, U, 3, U, U, 2, 3, 3, 3, 4, 4, U, 3	TA-203, H6C-07, H6C-07, H6C-07, NCPGR-42, H6C-07, NCPGR-42, TA-113, H2B-061, STMS-21, H6C-07, H6C-07, H1B-17, H5G-01, TS-19, H6C-07	7, 6.6, 13.7, 12.8, 7.9, 7.4, 6.6, 5.5, 5.6, 11.6, 20.3, 6.5, 6.1, 9.1, 10.6	

Harvest index	U, 2, 3, 5, 3, 3, 4, 1, 1	TA-113, TR-58, H6C-07, H1F-21, H6C-07, H6C-07, H5G-01, TA-203, TA-1	11.4, 8.3, 14.4, 6.2, 8.4, 9.1, 6.3, 9.5, 6.3	Hamwieh et al. (2013)
Percentage of empty pods	U, 2, U, 1, U, 2	TA-113, H1O-06, SCOM, TA-1, H5E-02, H1O-06	9.8, 12.5, 5.1, 5.6, 8.3, 7.1	
Empty pods	3, U, 3, 2, 3	GA-119, TS-19, TR-50 s, H1F-22, GA-6	12.7, 6.9, 6.9, 6.2, 5.6	
Seed number	3, U, 3, 4, 3, 3	H6C-07, H5E-02, H1B-04, H1B-17, H3G-09, H6C-07	14, 5.7, 6.5, 5.8, 7.1, 7.5	
Biological yield	U, 7, 3, 3, U	H5E-02, NCPGR-33, H3G-09, TR-31, TS-19	7.6, 5.6, 8.8, 8.6, 7.3	
Root weight	4, 4, 4	ICCM0249, TAA170, STMS11	58.20, 8.20, 58.20	Varshney et al. (2013a)
Root length density	4	NCPGR127–NCPGR21 A	10.90	Varshney et al. (2014a)
Root surface area	6, 4	TA106–H1I16, TAA170–NCPGR21 A	10.26, 16.67	
Shoot dry weight	4	TAA170–NCPGR21 A	17.59	
Plant height	3, 3, 4, 4, 8	TA34–NCPGR49, TA34–NCPGR49, NCPGR127–CPGR21A, NCPGR127–NCPGR21A, NCPGR164–CaM2187	10.00, 10.00, 30.20, 30.20, 14.73	
Days to 50% flowering	4, 8	NCPGR127–TAA170[A], NCPGR164–CaM1918	24.49, 26.87	
Days to maturity	6, 8, 4	TA106–CaM0399, NCPGR164–CaM1918, NCPGR127-TAA170A	12.13, 18.83, 19.71	
Pods per plant	4	NCPGR127–NCPGR21	23.18	
Seeds per pod	4	TAA170–NCPGR21 A	42.07	
100-seed weight	1, 4	NCPGR184–ICCM0009b, NCPGR127–NCPGR21 A	10.31, 58.20	
Biomass	4, 8	NCPGR127–NCPGR21 B, NCPGR164–CaM1918	21.32, 10.95	
Harvest index	4, 1, 1	TAA170–NCPGR21 B, cpPb-679915–CaM0393, NCPGR184–ICCM0009b	11.69, 14.36, 10.67	
Yield	1, 4	NCPGR136–CaM0046, TAA170–NCPGR21 A	13.98, 15.72	
Drought tolerance index	1	cpPb-679915–CaM0046	11.23	
SNP marker				
Drought tolerance index	1	cpPb-679915–CaM0046	11.23	Thudi et al. (2014b)
Root length density	4	NCPGR127–NCPGR21 A	10.90	
Root surface area	6	TA106–H1I16	10.26	
Root/shoot ratio	4	TAA170–NCPGR21 A	16.67	

(continued)

Table 4 (continued)

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
Root length density	4	ICCM0065–Ca4_11276225	10.65–12.09	Jaganathan et al. (2015)
Root surfacearea	4	Ca4_13840227–NCPGR	11.04	
Root dry weight	4	Ca4_13840227–NCPGR	10.85–13.56	
Shoot dry weight	4	Ca4_13840227–TAA170	13.89–17.59	
Plant height	4	Ca4_12982420–TAA170	10.78–26.91	
Primary branches	8	CaM0812–NCPGR164	10.05–34.57	
Days to 50% flowering	4	NCPGR164–Ca8_3050452	112.92	
Days to maturity	7	NCPGR164–Ca8_3050452	10.11–47.43	
100-seed weight	4	Ca4_13687456–TAA	10.12–60.41	
Biomass	4	NCPGR164–Ca8_3050452	10.11–16.63	
Harvest index	8	NCPGR164–Ca8_3050452	10.14–25.94	
Pods/plant	8	Ca4_13687456–TAA17	10.73–32.34	
Seeds/pod	4	–	11.09–45.40	
Yield	4	–	11.67–18.64	
Drought susceptibility index	4	–	13.00	
Drought tolerance index	4	–	10.10–10.76	
Root length density	4	bin_4_13239546–bin_4_13378761	10.36	Kale et al. (2015)
Root dry weight/total plant dry weight	4	bin_4_13393647–bin_4_13547009	20.09	
shoot dry weight	4	bin_4_13393647–bin_4_13547009	25.22	
Plant height	4	bin_4_13239546–bin_4_13378761	41.76	
Primary branches	8	bin_8_6034209–bin_8_5984553	11.27	
Days to 50% flowering	8	bin_8_6034209–bin_8_5984553	44.76	
Days to maturity	7	bin_7_12870961–bin_7_12856579	45.38	
100-seed weight	4	bin_4_13239546–bin_4_13378761	59.83	

Delta carbon ratio	4	bin_4_13239546–bin_4_13378761	11.90	
Harvest index	8	bin_8_6034209–bin_8_5984553	15.42	
Pods/plant	4	bin_4_13239546–bin_4_13378761	16.66	
Plant vigor	4	Bin_4_13239546-Bin_4_13378761	–	Sivasakthi et al. (2018)
3D-leaf area	1, 3, 4, 4, 4, 6, 5,	Bin_1_16247689-Bin_1_16256900, Bin_3_38153326-Bin_3_38067857, Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_6_59002540-Bin_6_45589823, Bin_5_7175460–Bin_5_5089713	53, 5, 11, 12, 19, 6	
Projected leaf area	4, 4, 4	Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_4_13048918-Bin_4_13097584	8, 9, 11	
Plant height	1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 4, 4, 4, 4, 5, 7, 7, 7, 7, 5, 7, 7, 7, 7	Bin_1_46320971-Bin_1_46550755, Bin_1_4992190–Bin_1_4870121, Bin_2_6254554–Bin_2_9665288, Bin_2_30506636-Bin_2_30527477, Bin_2_30506636-Bin_2_30527477, Bin_2_6254554–Bin_2_9665288, Bin_2_6254554–Bin_2_9665288, Bin_3_33718067-Bin_3_33467733, Bin_3_18480135-Bin_3_18295791, Bin_3_18480135-Bin_3_18295791, Bin_4_18035954-Bin_4_18295099, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_5_31281006-Bin_5_31262177, Bin_7_1934083–Bin_7_1901490, Bin_7_2149821–Bin_7_2075414, Bin_7_12083724-Bin_7_11984393, Bin_7_12083724-Bin_7_11984393	7, 5, 9, 6, 6, 10, 11, 8, 9, 9, 5, 34, 36, 39, 5, 7, 6, 10, 11	
Plant height growth rate	4, 4, 4, 4, 7	Bin_4_3535309–Bin_4_3596208, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_7_2149821–Bin_7_2075414	4, 13, 23, 23, 10	
Shoot dry weight	4, 4, 5, 5	Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_5_42970924-Bin_5_43185799, Bin_5_24533589-Bin_5_20895327	9, 18, 5, 6	
Specific leaf area	3, 4, 4, 4	Bin_3_33718067-Bin_3_33467733, Bin_4_12586721-Bin_4_12650792, Bin_4_16602407-Bin_4_16662055, Bin_4_12586721-Bin_4_12650792	7, 6, 4, 8	
Specific leaf weight	5, 1, 4, 4, 4	Bin_5_31507607-Bin_5_31449929, Bin_1_9838522–Bin_1_9855212, Bin_4_48635948-Bin_4_48732122, Bin_4_48635948-Bin_4_48732122, Bin_4_13393647-Bin_4_13547009	5, 5, 5, 4, 11	

(continued)

Table 4 (continued)

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
Evapotranspiration	4, 4, 5, 6	Bin_4_11992806-Bin_4_12061305, Bin_4_12586721-Bin_4_12650792, Bin_5_41290102-Bin_5_41678447, Bin_6_55893751-Bin_6_54270280	6, 7, 5, 5	
Evapotranspiration rate	3, 4, 4, 4, 7	Bin_3_29064360-Bin_3_28987359, Bin_4_47733094-Bin_4_47796904, Bin_4_10416329-Bin_4_10421332, Bin_4_11210420-Bin_4_11690035, Bin_7_32532047-Bin_7_32710042	8, 5, 8, 11, 5	
Transpiration	4, 4, 5, 1, 1, 2, 3, 7,	Bin_4_12321889-Bin_4_12331005, Bin_4_12321889-Bin_4_12331005, Bin_5_43185799-Bin_5_43439397, Bin_1_45932222-Bin_1_45934626, Bin_1_10100492-Bin_1_10111521, Bin_2_2241242–Bin_2_4309038, Bin_3_29064360-Bin_3_28987359, Bin_7_19452801-Bin_7_16741421	9, 8, 5, 5, 5, 5, 10, 5	
Plant vigor	1, 3, 3, 3, 4, 4, 4, 4	ICCM0009a-STMS21, NCPGR268-NCPGR255, CaM1024-NCPGR49, ICCeM050-TS58s, H4G11-TR20, ICCM0249-NCPGR127, TAA170-NCPGR21, GA24-STMS11	3, 3, 5, 4, 3, 7, 44, 13, 4, 10	
3D-leaf area growth rate	8, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4,	CaM1918-NCPGR170, NCPGR127-TAA170, TAA170-NCPGR21, GA24-STMS11, TAA170-NCPGR21, TR11-GA24, CaM1903-ICCM0249, TAA170-NCPGR21, TR11-GA24, TR11-GA24, TR11-GA24	10, 14, 18, 8, 23, 11, 5, 6, 6, 8, 8	
Projected leaf area	4, 4, 4, 4, 4, 4, 4	CaM1903-ICCM0249, NCPGR127-TAA170, TAA170-NCPGR21, GA24-STMS11, ICCM0249-NCPGR127, TR11-GA24, TAA170-NCPGR21	9, 12, 13, 7, 5, 6, 13	
Leaf area growth rate	4, 7	TAA170-NCPGR21, CaCISP117-STMS12	7, 5	
Plant height	1, 1, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 6, 6, 6, 6, 6, 7, 7, 7, 7, 7, 7, 7, 8, 8, 8	ICCM0009a-STMS21, cpPb-490962-cpPb-677672, ICCM0249-NCPGR127, TAA170-NCPGR21, TR11-GA24, ICCM0249-NCPGR127, TAA170-NCPGR21, GA24-STMS11, GA24-STMS11x, TAA170-NCPGR21, TR11-GA24, TA120-CaM0389, TR7-CaM1790, NCPGR202-CaM1760, NCPGR4-TA106, ICCeM051-TR7, NCPGR202-CaM1760, CaM0111-NCPGR99, TA76s-TR24, CaM2155-CaM0111, TA76s-TA142, CaM2155-CaM0111, TA180-ICCM197a, TA76s-TA142, CaM1918-NCPGR170, H1H14-TA25, H1H14-TA25	8, 4, 5, 23, 9, 10, 36, 15, 8, 32, 14, 5, 5, 4, 4, 4, 3, 6, 4, 6, 5, 5, 4, 5, 3, 3, 3	
Plant height growth rate	4, 4, 4, 4, 4, 4, 4, 8, 8	TR11-GA24, TAA170-NCPGR21, TAA170-NCPGR21, GA24-STMS11, ICCM0249-NCPGR127, TAA170-NCPGR21, TR11-GA24, CaM1918-NCPGR170, CaM1918-NCPGR170	9, 11, 20, 12, 4, 25, 14, 4, 5	

R-3D/PLA	1, 1, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 6, 6, 6, 6, 6, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	cpPb-677672-cpPb677690, cpPb-677672-cpPb677690, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM1684-H1A19, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM1903-ICCM0249, TAA170-NCPGR21, STMS2-NCPGR93, TA106-H1I16, NCPGR4-TA106, TA106-H1I16, TA106-H1I16, GA26-CaM0399, NCPGR19-CaM2041, cpPb-490874-CaCISP117, TA180-ICCM197a, NCPGR19-CaM2041, CaM1417-ICCM199c, CaCISP117-STMS12, CaM1942-CaM2155, NCPGR19-CaM2041, CaCISP117-STMS12, CaM0111-NCPGR99	6, 5, 11, 12, 13, 11, 9, 15, 13, 10, 5, 4, 8, 6, 7, 10, 10, 6, 10, 4, 6, 7, 7, 4, 6, 9, 5	
Shoot dry weight	4, 4, 4, 4, 4, 4, 4	TR11-GA24, TAA170-NCPGR21, ICCM0249-NCPGR127, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, NCPGR21-TR11	8, 11, 6, 11, 11, 20, 10	
Specific leaf area	4, 4, 4, 6, 7	TAA170-NCPGR21, ICCM0249-NCPGR127, NCPGR127-TAA170, NCPGR202-CaM1760, ICCM197a-CaM1567	5, 7, 8, 7, 7	
Specific leaf weight	3, 4, 4, 4	TR2-NCPGR10, TAA170-NCPGR21, NCPGR127-TAA170, TAA170-NCPGR21	5, 7, 5, 7	
Leaf area index	2, 4, 4, 4, 4, 4, 4	H1P092-CaM0142, CaM1903-ICCM0249, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170	5, 5, 9, 7, 9, 10, 16	
Evapotranspiration	4, 4, 4, 4, 6, 7	CaM1903-ICCM0249, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, H4H06-NCPGR200, CaCISP117-STMS12	6, 8, 7, 11, 6, 6	
Evapotranspiration rate	4, 4, 4, 4, 4, 7, 7, 7	NCPGR127-TAA170, NCPGR21-TR11, STMS11-cpPb680552, NCPGR21-TR11, STMS11-cpPb680552, TA142-TR24, CaM1567-NCPGR141, TA142-TR24	8, 5, 10, 6, 11, 6, 7	
Transpiration	4, 4, 4, 5, 5, 6, 7, 8, 8	ICCM0249-NCPGR127, NCPGR127-TAA170, cpPb-322921-TA132, cpPb-682328–NCPGR189, cpPb-682328–NCPGR189, CaM1125-H1F21, TA180-ICCM197a, CaM2187–CaM1918, NCPGR164-CaM2187	7, 12, 4, 8, 11, 4, 8, 7, 14	
Transpiration rate	7, 7, 7, 7, 7	NCPGR19–CaM2041, TA142-TR24, NCPGR19-CaM2041, NCPGR19-CaM2041, NCPGR19-CaM2041	11, 7, 10, 10, 10	

drought stress conditions was reported in RILs (recombinant-inbred-lines) of chickpea (Chandra et al. 2004). These RILs were developed from a cross between ICC-4958 (a drought-tolerant chickpea cultivar with deep roots and high biomass) and Annigeri (a drought-susceptible chickpea genotype). Although only 14 SSR markers were used to identify the marker-trait associations for root dry weight, shoot dry weight, and root length, this study described the usefulness of statistical models to identify the QTL-linked markers without a linkage map. Later, another study identified 15 genomic regions for various traits under terminal drought conditions by using 97 SSR markers (Rehman et al. 2011). These regions were identified from a RIL population, developed from a cross of drought-tolerant (ILC 588) and susceptible (ILC 3279) genotypes that were phenotyped for 2 consecutive years across two locations. Stomatal conductance and canopy temperature were the most vital traits associated with drought tolerance. Stomatal conductance and canopy temperature had three and six QTLs respectively and the range of phenotypic variation was 7–15%. These regions can be potentially used in a breeding program for the development of drought-tolerant chickpea genotypes.

Another RIL population of 181 lines developed by a cross of ILC5889 and ILC3279 was used for the identification of drought-linked QTLs by using 77 SSR markers (Hamwieh et al. 2013). The evaluation of these lines was done in ten environments across three different locations under different drought treatment levels. As a result, 93 genomic regions associated with 12 drought-tolerant-related traits (plant height, days to flowering, maturity, etc.) were identified in these RIL populations of chickpea. The QTLs associated with days to flowering had maximum phenotypic variation i.e. 24%. After pooling the data of QTLs obtained from drought and irrigated environments, it was observed that the QTLs of the drought resistance were significantly expressed under drought stress, whereas they had no strong expression under normal well-watered conditions. The very highest contribution from the allele "A" of marker H6C07 was reported as 80% and 29.8% for late planting and drought stress, respectively. That range of the contribution from a single allele is a much higher amount that can be used for the development of drought-tolerant chickpea genotypes.

Another study used 82 different molecular markers (19 ISSR, 28 RAPD, 38 STMS) for the identification of QTLs linked to drought tolerance, by using an intraspecific $F_{2:3}$ population developed from ILC32799 and ICCV2 (Jamalabadi et al. 2013). Among these markers, only 52 were finally mapped on the eight linkage groups. Morphological traits such as plant height, days to flowering, and 100-seed weight were phenotypically evaluated against drought stress. ISSR and RAPD markers exhibited the high segregation distortion as compared with STMS markers. Similarly, 26 of 82 markers were unlinked, and among these markers, the most common were ISSR and RAPD. The phenotypic variation was 32%, 29%, and 51% for QTLs associated with days-to-flowering, plant height, and 100-seed weight, respectively. Similarly, the RIL population of desi chickpea (ICC4958 × ICC1882) was used for the identification of a QTL-hotspot, associated with a deep root system under drought stress (Varshney et al. 2013a). QTL mapping revealed the association of three SSR markers, namely, TAA170, ICCM0249, and STMS11, with the QTL-

hotspot region. That QTL-hotspot region was found in linkage group-4, linked with root traits, and had shown 58.20% explained phenotypic variation.

Advances in genome sequencing techniques, such as NGS, provide easier and cheaper ways to sequence the genome. Thus, QTL mapping by using high-throughput sequencing tools has been shifting toward precise and quick markers such as SNPs. In the chickpea, association mapping was used for the identification of drought-related QTLs by using 300 diverse accessions (Thudi et al. 2014b). The distribution of diversity array technology (DArT) markers was equal across the genome of chickpea. These markers were used to explain the population structure, and three subpopulations were recognized by means of the admixture model in STRUCTURE. Association mapping was performed by using 1872 markers proportionally divided as 36 SSR, 113 gene-based SNPs, 651 SNPs, and 1072 DArTs. Subsequently, 312 marker-trait associations (MTAs) were recognized, the highest number of MTAs being 70, associated with 100-seed weight. The number of identified SNPs was 18, recognized from five different genes and associated with the drought-tolerant traits. The identified MTAs were the significant and potential source for the development of drought tolerant chickpea by improving the traits associated with these MTAs.

Later, a study was conducted by using two populations, ICCrIl03 (ICC 4958 × ICC1882) and ICCrIl04 (ICC 283 × ICC 8261), and subjected to phenotypic screening against drought stress by using 20 different yield- and drought-related traits for seven seasons across five different locations in India (Varshney et al. 2014b). Different type of QTLs, main-effect QTLs (45), epistatic QTLs (973), and drought tolerance-linked QTLs (9), were identified from these populations. One cluster had 48% of robust main-effect QTLs associated with 12 parameters. That cluster was present on the CaLG04, explaining 58.20% of phenotypic variation, and defined as "QTL-hotspot." This genomic region had seven SSR markers associated with drought stress, and the introgression of that genomic region into the chickpea accession would be effective for a breeding program.

On the other hand, bacterial artificial chromosome (BAC) libraries were developed to construct the physical map of chickpea against drought-stress (Varshney et al. 2014a). Two genetic maps, associated with the physical map, were developed by using SSR markers and derived through BAC-end sequencing. Of 337 BES-SSRs, 259 markers were used for the genetic map and integrated into three populations, one inter-specific and two intra-specific mapping populations. The number of identified QTLs was 654 in the QTL hotspot region linked with drought tolerance.

Moreover, this already identified QTL-hotspot on CaLG04 was further explored for the identification of drought-linked genomic regions by using the advanced sequencing tool, genotyping by sequencing (GBS) in a chickpea RIL population (ICC 4958 × ICC 1882) (Jaganathan et al. 2015). The RIL population were phenotyped for 20 drought-related traits within 7 years. Through GBS, data were generated from the parent (ICC-4958, 6.24 Gb, and ICC-188, 25.65 Gb) and RILs population (59.03 Gb), and 828 unique SNPs were identified for the genetic map. A QTL hotspot was found with 49 SNP markers harboring drought tolerance. Cumulatively, 164 main-effect QTLs with 24 unique QTLs were also identified in

the hotspot. The identified SNPs were also converted into cleaved amplified polymorphic sequence (CAPS) and derived CAPS (dCAPS) markers. The markers can enhance the efficiency of marker-assisted breeding in chikcpea.

The same QTL-hotspot (CaLG04) region was further explored by using the two approaches, QTL and gene enrichment analysis among 232 RILs, developed by the single-seed-descent method (ICC-4958 × ICC-1882) within five seasons and across the five locations in chickpea (Kale et al. 2015). QTL identification was done for 17-drought-related traits along with two drought-tolerance indices. A total of 53,523 SNPs were identified from RILs, and these SNPs were used for the construction of a high-density bin map. Gene enrichment analysis based on SNPs associated with the drought-related traits had shown enrichment for 23 genes on the hotspot region. Only 12 genes were common in both approaches and functionally validated by qRT-PCR, resulting in the identification of four promising candidate genes, present in the QTL hotspot.

Quite recently, this QTL hotspot (CaLG04) was further analyzed for the identification of QTLs associated with drought tolerance by using a 232 RILs population (ICC-4958 × ICC-1882) (Sivasakthi et al. 2018). Canopy conductance and plant vigor had 21 major QTLs (M-QTLs), identified with the help of an ultra-high-density bin map. CaLG04 had 13 M-QTLs, linked with canopy conductance, and had favorable alleles from a high vigor parent (ICC-4958). Another M-QTL was also identified on the CaLG03, linked with canopy conductance. Comparative analysis of the QTLs showed that by increasing the marker density, QTL size was reduced while phenotypic variation percentage increased markedly.

Thus, genomic resources are the efficient and comprehensive way to target the genomic regions linked with tolerance to stress, such as drought stress. Identified QTLs can be used in marker-assisted breeding for the development of drought tolerance in the chickpea.

3.2.2 Transcriptomic Resources

Transcriptomics is the study of the transcriptomes, which are generated by the genome, under different environments in the cell, using high-throughput systems such as RNA-Seq and microarray analysis. Comparison of transcriptomes provides a platform for the identification of genes that are differentially expressed in diverse cell populations, or in answer to changed treatments. Transcriptomic resources are the sources used to make the plant expression profile under different environmental conditions, based on their mRNA/cDNA study. Principally, the transcriptomic study was facilitated with the help of the RNA-Seq technique. RNA-Seq is a cost-effective approach, accelerated by the invention of sequencing tools, such as NGS (Wang et al. 2009). Differentially expressed genes and their isoforms and variants such as SNPs, SSR, and InDels can be identified through transcriptomic dissection of genes (Zhao et al. 2014). The use of transcriptomics study in crop plants is a cyclic process that involves the identification of connective genes and linked pathways and provides further support for gene cloning, evaluation, and development of large-scale

genetic markers. Transcriptomic analysis through ESTs is a very old method, used to develop the transcriptome of chickpea under varying environmental conditions.

ESTs were used to compare the responses of chickpea genotypes against drought stress and also subjected to find some up-regulated and down-regulated genes (Gao et al. 2008). cDNA libraries were used to develop clones, and almost 2500 clones were selected randomly from each cDNA library. The selected clones were subjected to sequencing and 92 genes were identified which had differential expressions. Among these genes, the number of up- and downregulated genes were 36 and 56, respectively, under a stressed environment. These upregulated genes were classified into four major groups, metabolism-related genes (7), genetic information-processing genes (1), cellular-processing genes (1), and stress-related genes (27), among their groups. The expression of these genes was also associated with lipid transfer proteins (LTPs), late embryogenesis abundant (LEA) proteins, rubisco-encoding genes, and chlorophyll-binding proteins (*a*/*b*) under drought stress as well. This study provides the platform for understanding of the molecular basis of drought stress in chickpea.

Another study was conducted for the generation and evaluation of ESTs along with gene-based markers in chickpea against drought and salt stress (Varshney et al. 2009). This study identified a total of 20,162 new ESTs, among them 6404 unigenes, portioned as 11,904 and 2595 ESTs against drought and salt stress libraries of root tissues, respectively. Based on 177 SSR markers and 742 genes with SNPs in chickpea, the transcriptomic map of chickpea became comprehensive and more informative. The molecular markers developed from transcriptomic and ESTs data, were used to facilitate direct moving toward the target genes. Similarly, two contrasting chickpea genotypes were used for transcriptomic analysis based on ESTs, obtained from cDNA libraries, and developed from different time points (Jain and Chattopadhyay 2010). A total of 319 ESTs were obtained from different cDNA libraries and were classified into 11 clusters based on their expression profile. Based on higher expression of ESTs under drought stress in tolerant cultivars, 53 ESTs were selected and subjected to further screening analysis. These highly expressed ESTs were involved in protein metabolism, transcription, signal transduction, and cellular organization. These ESTs were the source for improving drought tolerance in chickpea by targeting beneficial genes as identified from a tolerant cultivar.

The suppression subtraction hybridization (SSH) method was used to generate ESTs libraries from the root and shoot tissues of two contrasting genotypes under terminal drought stress by using a dry-down experiment in chickpea (Deokar et al. 2011). Based on the results, a total of 5494 high-quality ESTs were drought responsive. The number of terminal-drought responsive unigenes was 1500. Similarly, 830 unigenes were only expressed in roots under terminal drought stress that showed the presence of genotype-specific expression among contrasting genotypes. On the other hand, pyrosequencing technology was used for the transcriptomic analysis of chickpea under drought stress (Garg et al. 2011). By using this technique, two million high-quality sequences were generated with an average length of 372 bp. Based on de novo assembly, it was clearly indicated that the hybrid assembly of short-read and long-read assemblies revealed better results. More than 4000 SSR markers were

identified and used as functional molecular markers in chickpea. Finally, based on the resultant data, a web resource, namely, the Chickpea Transcriptome Database (CTDB), was developed and made publicly available. So, this study was the source for accelerating the genomic research and breeding programs against drought stress in chickpea.

In parallel, super-SAGE (serial analysis of gene expression) analysis of gene expression was used in chickpea against drought stress by using root tissues (Molina et al. 2008). Super-SAGE is considered as an advanced technique of the SAGE. It is used to create a genome-wide superior-quality transcriptome profile of the chickpea against drought stress. Super-SAGE was used to define cDNA positions by producing 26-bp-long fragments (26-bp tags). Based on this information, mRNA sequencing information was clearly characterized. A total of 7532 UniTags were more than 2.7-fold differentially expressed and 880 were regulated more than eightfold under drought stress as compared to normal irrigated conditions. The genes associated with photosynthesis and energy metabolism were downregulated. On the other hand, transcription factors and signal transduction-related genes were down- and upregulated under drought stress. Moreover, Super-SAGE tags were applied to develope microarrays and probes for RT-PCR, thus overcoming the deficiency of genomic techniques in non-model plants as well.

Quite recently, the cDNA-AFL methodology was used to evaluate the chickpea genotypes for drought stress (Mozafari et al. 2018). About 295 transcript-derived fragments (TDFs) were identified under drought stress. cDNA was subjected to sequencing and classified into different groups related to macromolecule metabolism, signal transduction, cellular transport, cell division, energy production, and transcriptional regulation under different levels of drought stress. Based on transcriptomic results, the genes associated with transcription of mitochondrial chaperone, hydrolases, ribosomal protein S_8, NADPH dehydrogenase, histone deacetylase, calmodulin, histone deacetylase, and chloride channels were significantly affected under drought stress.

Later, the use of microarray for transcriptomic study in chickpea had become common. Leaf and root tissues of chickpea were used for transcriptomic study against drought stress by using an oligonucleotide microarray (Wang et al. 2012). A total of 6164 oligonucleotides spotted microarray was constructed by using 36,301 ESTs as well as 283 sequences of nucleotides. Based on temporal gene expression, the number of differentially expressed unigenes was 2623 and 3969 in root and shoot tissues, respectively. Further, 110 drought-responsive pathways were identified. Similar to other findings, the number of expressed genes under a stressed environment remained greater; in the current study, the number of expressed genes under drought stress was more as compared with normal, 88 and 52 in root and shoot tissues, respectively. More genes were found to be expressed in leaves as compared to roots, linked with different biological activities under drought stress. Another study was conducted to examine the transcriptome dynamics in chickpea using microarray, by applying drought stress and *Ralstonia solanacearum* infection (Sinha et al. 2017). *R. solanacearum* is the chickpea pathogen responsible for wilt disease. The drought-stressed plant was infected with the pathogen for 2 days

(short duration) or 4 days (long duration). The number of differentially expressed genes were 821 and 1039, respectively, under the short and long duration of stressed environment. The pathogen also had a cumulative effect on the drought stress, thus mimicking a combined stress effect. Most of the genes were found upregulated under infection by the pathogen. Real-time PCR was used to validate the microarray results of differentially expressed genes under drought and pathogen stress. This transcriptome is the way to target the resistant and desired genes against these stresses.

After microarray, because of the presence of NGS tools, transcriptomics has been shifted toward RNA-Seq. It also is known as whole transcriptome shotgun sequencing (WTSS), used to show the quantity and quality of RNA exhibited in a biological tissue at a given time point. Principally, it is concerned with the alternative gene spliced transcripts, mutations/SNPs, gene fusion, posttranscriptional modifications, and differential gene expression under varying environments (Wang et al. 2009). So, in chickpea, the transcriptomic profile was made by using root and shoot tissues against drought, salt, and cold stresses (Garg et al. 2015). A total 250 million of excellence reads from stressed and nonstressed tissues were generated. Among the identified transcripts, 11,640 transcripts were seen to be present at least one of the applied stress environments, whereas 3536 transcripts were identified through reference-based transcriptomic assembly, differentially expressed in response to abiotic stresses. Some genes were found to be involved in the regulation of the RNA metabolic process, posttranslational modifications, and epigenetic regulation. The resultant transcriptome profiling of chickpea is the key source of various plant responses to stresses and open avenues to conduct applied and functional genomic studies for improving stress tolerance in chickpea.

Similarly, root and shoot tissues of chickpea were used for RNA-Seq. against drought and salt stress at both stages, vegetative and reproductive (Garg et al. 2016). An Illumina HiSeq 2000 platform was used for sequencing of libraries to generate more than 30 million 100-bp-long paired-end reads for given samples. Differentially expressed genes were identified by using Cuffdiff. There were 4954 and 5545 genes among drought-tolerant and salt tolerant genotypes, respectively. The regulatory network linked with drought and salinity stress tolerance was the key findings of the transcriptomic dynamics. Further, RNA sequencing was also performed to analyze the genes/pathways linked with tolerance/susceptibility against drought stress in chickpea by using two contrasting genotypes: ICC-283 (drought tolerant) and ICC-8261 (drought sensitive) (Badhan et al. 2018). Many genes, such as MYB-related protein, alkane hydroxylase MAH-like, ethylene response, xyloglucan endotransglucosylase, cysteine-rich, BON-1 associated, peroxidase 3, vignain, transmembrane domain, and mitochondrial uncoupling, were upregulated under drought stress in the tolerant genotypes whereas some other genes were downregulated in the sensitive genotypes at the same time point. RNA profiling of the tolerant genotype is a good source for the genetic donor to develop tolerance in the sensitive genotypes.

Similarly, the *Cicer arietinum* Gene Expression Atlas (CaGEA) was presented by using RNA-Seq analysis of chickpea (ICC-4958) under drought stress at different

growth stages (Kudapa et al. 2018). Differentially expressed genes identified from a pairwise combination of samples numbered 15,497. Root development, nodulation, flowering, and seed development processes varied significantly in terms of differential gene expression. The differential gene expression related to drought stress was validated against drought stress present in the QTL hotspot. Moreover, RNA-Seq was used to characterize two Kabuli chickpea genotypes under varying levels of drought stress at the time of early flowering (Mashaki et al. 2018). About 4572 differentially expressed genes were recognized. The number of genes related to drought tolerance was varied according to tissue type; root and shoot carried 261 and 169 genes, respectively. In tolerant genotypes, a gene ontology study was used to further sub-categorize chickpea based on different plant responses: defense response, response to stress, and stimulus–response. Many TFs were recognized, involved in different metabolic pathways, such as flavonoid, proline, and ABA biosynthesis. The QTL hotspot region was also explored for differential gene expression of candidate genes associated with drought stress. Finally, transcriptomic resources are the potential source in plant breeding for the development of drought-tolerant chickpea verities based on their transcriptome profile of drought-responsive candidate genes.

3.2.3 Proteomics

Focus on the application of proteome-wide profiling in plants for the characterization of phenotype has emerged gradually with the advances in genomic tools. In proteomics, the most common techniques are two-dimensional (2-DE) polyacrylamide gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The use of liquid chromatography for proteomic analysis is also becoming progressively popular (Komatsu et al. 2013). Omics-based assisted breeding through protein-based markers immensely expands its worth for the improvement of plant breeding. Proteomics simply assist the plant breeder to advance knowledge regarding the investigation and identification of complex stress mechanisms in plants under stress conditions (Eldakak et al. 2013).

Transcriptomic level does not have an exact or constant correlation with the protein functions and their abundance, altered by posttranscriptional modifications. Because of the need to develop the high-throughput proteome, with respect to developing drought-responsive novel proteins in plants, most of the studies to date about drought stress are mostly related to alterations in gene expression whereas very little information about their products has been available until recently. Nevertheless, the worth of drought-responsive genes is incomplete without information about their functions. So, to expose the plasticity of gene expression with respect to their products, proteomics analyses are necessary, because this enables us to visualize the physiological position of the cell by observing protein formation. On the other hand, different factors such as protein abundance, electrophoretic properties, protein abundance, and size are present, which are responsible for the

limiting synthesis of proteins (Westbrook et al. 2006). Proteomics is an important tool for understanding the tolerance mechanisms of the plant under stress conditions. It gives information about which type of protein is formed and what its function is under different environmental conditions.

In chickpea, the cell-wall proteome was developed to recognize the novel functions of extracellular proteins (Chattopadhyay et al. 2006). This proteome was proved as a platform for the comparative studies of these proteins under drought stress. Proteomic analyses discovered some new extracellular-matrix proteins of unknown functions vis-a-vis the existence of several known cell-wall proteins. Moreover, some unknown proteins with known chemical activities were recognized based on the proteome map of chickpea. Another study was carried out for the proteomic profiling of eight commercial varieties of chickpea against drought stress by using electrospray ionization time-of-flight mass spectrometry, the Agilent 1100 Series HPLC system with a Q-STAR Pulsar I mass spectrometer (Bhushan et al. 2007). Based on quantitate image analysis, 163 protein spots were shown that had seemed to be significantly changed according to their intensities by more than 2.5-fold during drought stress. Based on two-dimensional electrophoresis results, a total of 134 differentially expressed proteins were recognized under dehydration stress. A proteome profile revealed the possible functions of some known as well as unknown proteins against drought stress tolerance.

Moreover, in chickpea, the formation of new proteins in response to drought stress was reported in the tolerant genotypes by making the proteomic profile through SDS-PAGE (Patel and Hemantaranjan 2013). Among these proteins, the most common were dehydrin-responsive proteins (DRPs) found in the seeds of chickpea under drought stress. Similarly, the effect of abiotic stresses such as drought, salt, and heat was studied through the leaf proteome in chickpea (Santisree et al. 2017). A total of 590, 797, and 248 regulated proteins were found for drought, salt, and heat stress, respectively. Nitric oxide was applied as a foliar spray, and as a result many proteins were modulated to increase stress tolerance in chickpea. Signaling pathways and regulatory proteins responsible for stress tolerance had been identified in chickpea with the help of proteomic analysis. Various stress-related proteins such as ABRE, MYB, and MYC were recognized in chickpea for drought stress (Hussain et al. 2019).

In chickpea, iTRAQ-based proteomic analysis of mitochondrial proteins, responsible for drought adaptions, was performed (Gayen et al. 2019). A total of 40 drought-responsive proteins were found; their expressions were regulated by drought stress. Various metabolic pathways such as oxidative phosphorylation, pathways of carbon fixation, and the purine-thiamine metabolic network were regulated by differentially expressed proteins. The proteome delivers intriguing insights into the metabolic pathways and provides clues associated with drought tolerance in chickpea. Similarly, two species of chickpea, *C. arietinum* and *C. reticulatum*, were used for comparative physiological and proteomic analysis by exposing drought stress (Çevik et al. 2019). MALDI-TOF/TOF-MS/MS-based quantitate pruritic analysis identified 24 differentially expressed proteins in response to drought stress. *C. reticulatum* had better adaption to drought stress and showed upregulation of the

proteins that were involved in the energy mechanisms and photosynthesis against drought conditions. Moreover, proteins related to glutamine synthetase, sucrose and proline biosynthesis, and cytosolic fructose-bisphosphate aldolase were also upregulated in *C. reticulatum* under drought stress. This study provides clues for targeting the drought-responsive proteins in chickpea that were produced in *C. reticulatum*. Thus, a remarkable development in interrogating proteomes has shown its significance for the identification and evaluation of differential drought responses in plants understress. Although recent technologies have been used in the proteome that make it possible to study the changes in protein expression, yet the proteome profile of crop plants is very new.

3.2.4 Phenomics

Phenomics considers the phenotyping of the plant by using various tools for the measurement of morphological data. The complex traits such as drought tolerance are yet a challenge to measure. The combination of genetic and modern genomic techniques with breeding methodologies and precise phenotyping is considered effective for the understanding of metabolic pathways through which tolerant cultivars can be developed. Phenotyping is the vital phase before the usage of genetic and physiological strategies for enhancing drought tolerance in crop plants (Mir et al. 2012). Phenomics is an important technique that has been used for the identification and dissection of physiological mechanisms related to drought tolerance. Several techniques have been used for phenomics, such as spectroscopy and fluorescent microscopy to measure the rate of photosynthesis and to study photosynthetic processes. Transpiration and temperature profiles are recorded by infrared cameras as well as 3D cameras to record alterations in growth processes (Gupta and Rustgi 2004).

In chickpea, extensive studies on root-related traits were done for identification of drought-tolerant genotypes (Silim and Saxena 1993). The tolerant genotypes seemed to be those with an efficient and long root system as compared to susceptible genotypes under drought stress. Similarly, chickpea germplasm was grown under a low level of soil moisture had adverse effects in the form of terminal drought stress (Kashiwagi et al. 2005). So, the phenomics analysis of roots-related parameters is widely recommended to obtain useful results. It was recorded that the chickpea genotypes with more profuse and deeper root systems can extract more water from the deep water table and are considered as drought-tolerant cultivars. Molecular breeding, genetic dissection, and phenotyping have been used collectively to understand the mechanisms of drought tolerance in chickpea. Different drought-related traits including root, maturity, carbon assimilation, shoot biomass, and seed yield were targeted in chickpea. Phenotypic data were recorded to characterize the germplasm in response to drought stress (Upadhyaya et al. 2012). Moreover, 20 genotypes of chickpea Desi and Kabuli were screened based on indices against drought stress (Khan et al. 2018). Diverse results were obtained between chickpea genotypes. Two genotypes (NKC-5-S-20 and NKC-5-S-17) were found to be more drought tolerant in irrigated as well as rainfed areas. The seed yield of these genotypes remained healthy as compared with that of other genotypes under drought.

4 Genomics-Assisted Breeding

To cope with challenges caused by climate change, genomics-assisted breeding has been adopted successfully by using available genomic tools such as genetic maps and genetic markers. The latest sequencing tools (NGS) have been commonly used to sequence the genome with the contribution of different international institutes. Now, it has become possible to use genomics-assisted breeding for the development of chickpea genotypes to develop either tolerant or high-yielding varieties (Varshney et al. 2017). Genetic diversity, DNA fingerprinting of a plant genome, and the evolutionary relationship between chickpea relatives was studied by using DNA markers (Sudupak et al. 2002). Similarly, AFLP markers were used for the grouping of nine chickpea annual species, and that grouping was similar to RAPD markers (Shan et al. 2005).

Marker-assisted breeding is most commonly divided into two aspects: marker-assisted backcross breeding (MABC) and marker-assisted recurrent selection (MARS). MABC can be used to develop drought-tolerant accessions. Marker-assisted breeding is a rapid and comprehensive molecular breeding approach that is used to isolate the superior individuals and desired marker loci. For meaningful marker-assisted plant breeding, DNA markers should have a few key characteristics, such as quality and quantity, greater reliability, DNA polymorphisms, and low cost for assy designing (Mohler and Singrün 2004). In plant breeding, identification and characterization of QTLs is the key source for meaningful plant breeding to develop drought-tolerant plants. QTLs pyramiding strategy is also a feasible process for developing drought tolerance in plants (Luo et al. 2019).

Several studies based on marker-assisted breeding are reported in chickpea. Similarly, chickpea introgression lines were evaluated for drought tolerance, based on an QTL-hotspot obtained from the donor parent (drought-tolerant) (Sheoran et al. 2018). Based on marker analysis, the introgression lines had that *QTL-hotspot*, exhibited drought tolerance by making a good root system as compared to susceptible genotypes. Presence of the root-linked genomic regions as well as phenotypic resemblance with a recurrent parent was the indication of drought tolerance. Potential lines of chickpea were evaluated from a population of eight parents through multi-parent advanced generation inter-cross (MAGIC) (Samineni et al. 2017). Genetic diversity was created through MAGIC, used to develop promising lines of chickpea against drought stress. Thus, these introgression lines were recognized as drought tolerant as compared with some popular existing cultivars of chickpea.

On the other hand, MARS was exploited to develop elite lines of chickpea against drought stress. Identification of desired genes from genomic resources has become the supreme priority, to target genes related to stress or yield improvement (Samineni et al. 2017). In chickpea, a QTL-hotspot was obtained from a drought-tolerant chickpea line (ICC-4998) and transferred into two widely cultivated and adapted cultivars (JG-11 and Bharati) (Samineni et al. 2015). After transformation of the drought-linked genomic region, 20 introgression lines were developed and evaluated across the three to four locations. Several introgression lines had 10% higher yield than their parents because of better adaptivity under drought stress from a

different location under different environmental conditions, irrigated or rainfed. Moreover, that genomic region also had an influence on the other yield-contributing traits, seed size along with resistance to drought. The resultant chickpea cultivars were thought to be effective for the breeding program against terminal drought stress in chickpea.

To overcome the pyramiding issue of complex traits, an alternative method of marker-assisted selection has been invented during recent years that is most commonly known as 'genomic selection' (Hayes and Goddard 2001). Total information that can be obtained through genetic markers is used to study the breeding worth of the crop plants. The complex traits can be analyzed easily by rendering the pyramiding complex in marker-assisted selection. Genomic selection is the best practice for the assortment of preferred parents for breeding strategies. It can minimize the cost and standard of breeding time cycle for variety development, which is why it has become more popular for plant breeders to hasten the breeding program (Crossa et al. 2014; Hayes et al. 2009). When the population size is large, and the trait has a low range of heritability, then at that point, genomic selection is more effective as compared to phenotypic selection. In chickpea, based on genomic selection, it was revealed that yield was low in rainfed areas as compared to irrigated areas (Jaganathan et al. 2015). Moreover, stress-resistant cultivars with a high potential of production and adaptation were developed through genomic selection in chickpea (Samineni et al. 2017).

A genetic linkage map is required to develop the association between phenotype (single-marker analysis, interval mapping, composite interval mapping) and a marker that confers the targeted genomic regions. Along with basic steps of QTL mapping, different kinds of segregating population have been developed: double haploid, F_2 generation, recombinant inbred line, and near-isogenic line. In this way, the genomic regions contributing to the drought stress were discovered and explored from the genome of plants (Singh et al. 2015), wherein the meta-QTL technique is the way to study the complex QTLs, such as drought-related QTLs, and several populations are screened across the various locations and environments in plants. Canopy conductance and plant vigor were improved through QTLs mapping in chickpea (Sivasakthi et al. 2018): QTLs linked with canopy conductance and plant vigor were transferred from a drought-tolerant chickpea variety to a susceptible variety.

Association mapping identifies QTLs from a diverse panel, based on genome-wide linkage disequilibrium, relevant phenotypes, and forms of genomic variants. In association mapping, there is no need to develop experimental populations resulting from planned crossings. Exotic diverse germplasm is the significant material for association mapping (Mitchell-Olds 2010). In chickpea, 1872 markers were used for 300 diverse chickpea genotypes against drought-stress and market trait associations that were evaluated through association mapping among these genotypes (Thudi et al. 2014a). Similarly, chickpea genotypes were evaluated for drought tolerance by using phenotypic and molecular approaches (Sachdeva et al. 2018). In all, 90 alleles were identified, and polymorphism information content varied from 0.155 to 0.782 per locus. This information was used to detect tolerant and drought-prone genotypes (Sachdeva et al. 2018). In chickpea, four genetic

regions were identified, comprising different SNPs, which indicate the pleiotropic effects of genes under drought stress (Li et al. 2018). Notably, marker-assisted breeding was recognized as more efficient and accurate breeding as compared with conventional breeding. The exploration of germplasm through DNA markers shows profound impacts on conventional breeding. Thus, conventional and omic-based breeding have their relative significance in plant breeding, and the integration between these approaches is helpful for the improvement of drought tolerance in chickpea.

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