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Molecular and Morphophysiological Analysis of Drought Stress in Plants

Summy Yadav and Kamal Dutt Sharma

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<http://dx.doi.org/10.5772/65246>

Abstract

Drought is a major environmental stress factor that affects the growth and development of plants. Most of the physiological traits associated with drought tolerance are quantitative in nature. An important research strategy that has been widely used to deal with such complexity is to use molecular markers to identify quantitative trait loci (QTLs) in appropriate mapping populations. In response to drought brought about by soil water deficit, plants can exhibit either drought escape or drought resistance mechanisms, with resistance further classified into drought avoidance and drought tolerance. Drought escape is the ability of plants to complete the life cycle before severe stress arrives. Drought avoidance is the maintenance of high tissue water potential in spite of soil water deficit. Drought avoidance is consequence of improved water uptake under stress and the capacity of plant cells to hold acquired water that reduces water loss. Drought tolerance is the ability to withstand water deficit with low tissue water potential. Plant water status that includes leaf water potential, osmotic potential and relative water content (RWC) represents an easy measure of water deficit and provides best sensor for stress. Genomics-assisted breeding (GAB) approaches, such as marker-assisted selection (MAS), can greatly improve precision and efficiency of selection in crop breeding. Molecular markers can facilitate indirect selection for traits that are difficult or inconvenient to score directly, pyramiding genes from different sources and combining resistance to multiple stresses. Conventional breeding for developing drought-tolerant crop varieties is time-consuming and labor intensive due to the quantitative nature of drought tolerance and difficulties in selection for drought tolerance. The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection (MAS). This requires integration of knowledge from plant physiology and biotechnology into plant breeding. The availability of a large number of molecular markers, dense genetic maps and markers associated with traits and transcriptomics resources have made it possible to integrate genomics technologies into chickpea improvement.

Keywords: abiotic stress, drought, physiological traits, conventional breeding, marker-assisted selection, QTLs

1. Introduction

Plant growth and productivity is adversely affected by nature's wrath in the form of various biotic and abiotic stress factors. Water deficit is one of the major abiotic stresses, which adversely affects crop growth and yield. Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Strain is any physical and chemical change produced by a stress [1]. Stress is used with various meanings, the physiological definition and appropriate term as responses in different environmental situations. If a factor deviates from its optimum does not necessarily results in stress. Stress is a constraint or unpredictable change imposed on regular metabolic patterns of growth results in injury, disease or aberrant physiology. Plants are mainly exposed to stresses such as drought, precipitation, salt, flooding, heat, oxidative stress and heavy metal toxicity. Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation due to increase in temperature in nature. Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species [2]. Conventional plant breeding attempts have changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability [3]. Abiotic stresses tolerance is a complex trait, due to the interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth at different developmental stages [4]. High yield potential under drought stress is the target of crop breeders. In many cases, high yield potential can contribute to yield in moderate stress environment [5]. Drought stress leads to stomatal closure and limitation of gas exchange. Desiccation is much more extensive loss of water, which can potentially lead to maximum disruption of metabolism and cell structure and finally stops enzyme catalyzed reactions [6,7]. Drought stress is characterized by reduction in water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in hampering photosynthesis, disturbing the overall metabolism and finally the necrosis of plant [8]. Water stress inhibits cell enlargement more as compared to cell division. Plant growth is reduced by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters [9]. A better understanding of the morphophysiological traits can be used to create new varieties of crops to obtain a better productivity under drought conditions [10]. The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth [11]. A fundamental part for making the crops stress tolerant is to understand plant responses to different drought stress environments [12].

In response to drought brought about by soil water deficit, plants can exhibit either drought escape or drought resistance mechanisms, with resistance further classified into drought

avoidance (maintenance of tissue water potential) and drought tolerance [13]. Drought stress is the ability of plants to complete the life cycle before severe stress conditions arise. Drought avoidance is the maintenance of high tissue water potential under a soil water deficit. Improved water uptake under stress and the capacity of plant cells to hold acquired water reduces water loss leading to drought avoidance. Drought tolerance is the ability to withstand water deficit with low tissue water potential. Plants respond to water deficit using mechanisms of avoidance by improved root traits and by reducing water loss through reduced epidermal (stomatal and cuticular) conductance, reduced radiation absorption, and reduced evaporative surface (leaf area). Drought tolerance is the ability to withstand water deficit with low tissue water potential [14]. Plants under drought stress may survive by, among other mechanisms, maintaining cell turgor and reducing evaporative water loss by accumulating compatible solutes [15]. In recent years, much molecular information has been generated on the response of plants to environmental stresses. Plants respond to environmental stresses such as drought by the induction of both regulatory and functional sets of genes [16, 17]. Very little is known about the early events in the perception of stress signals [18, 19]. The common stress signaling pathways have been distinguished into abscisic acid (ABA) dependent and ABA independent [20, 21]. Most of the key genes in these pathways have been identified, such as transcription factors belonging to the class of dehydration responsive element-binding protein (DREB)/C-repeat-binding factor (CBF), ABA-binding factor (ABF), Myelocytomatosis oncogene (MYC) and Myeloblastosis oncogene (MYB), including the identification of the stress-responsive cis-elements ABA-responsive element (ABRE) and dehydration responsive element (DRE). Downstream of the early signal perception events, signaling genes and molecules acting as secondary messengers have been identified, revealing the role of Ca^{+} and reactive oxygen species (ROS) as secondary messengers. These regulatory mechanisms induce downstream functional genes, which are needed to establish new cellular homeostasis that leads to drought tolerance and/or resistance.

Most of the physiological traits associated with drought tolerance are quantitative in nature. Genomics-assisted breeding (GAB) approaches, such as marker-assisted selection (MAS), can greatly improve precision and efficiency of selection in crop breeding [23]. Integration of genomics and breeding has a great potential for crop improvement. Molecular markers facilitate indirect selection for traits that are inconvenient to score directly (e.g., root traits, resistance to root knot nematodes), pyramiding genes from different sources (e.g., bringing together ascochyta blight resistance genes from different donors) and combining resistance to multiple stresses (e.g., resistance to fusarium wilt and ascochyta blight). Recent years have seen tremendous progress in the development of large scale genomic resources such as DNA-based molecular markers, comprehensive genetic maps, whole-genome transcription profiling techniques to identify genomic regions and genes underlying plant stress responses [24]. These genomic tools will be useful to understand and access the diversity conserved in ex situ germplasm collections for crop improvement [25]. Thus, an understanding of drought stress and water use in relation to plant growth is of importance for sustainable agriculture. Conventional breeding for developing drought-tolerant crop varieties is time-consuming and labor intensive due to the quantitative nature of drought tolerance and difficulties in selection for drought tolerance [26]. Mapping of different genomes has been of interest to identify genomic

locations of disease resistance genes and other yield-related traits. Isolation and validation of genes underlying the QTL/genes for the traits of interest is an essential step to determine gene function. QTLs for drought tolerance have been identified for major important crop species such as rice, maize, wheat, barley, sorghum, pearl millet, soybean and chickpea. These QTLs were identified for many important traits which include yield and yield-related traits under drought stress conditions, physiological responses including water-soluble carbohydrates, carbon isotope ratio, osmotic potential, chlorophyll content, flag leaf rolling index, grain carbon isotope discrimination, relative water content, leaf osmotic potential, osmotic adjustment, chlorophyll and chlorophyll fluorescence parameters to drought stress, flowering time, root traits. Major QTLs contribute to the traits with higher phenotypic variation. These QTLs, after validation in desired germplasm, can be used for introgressing drought tolerance from the donor genotypes into less drought-tolerant cultivars or breeding lines (recipient parents) avoiding transfer of undesirable or deleterious genes from the donors (linkage drag).

2. Drought stress improvement

Drought can be defined as below normal precipitation that limits plant productivity. Drought can be classified as either terminal or intermittent. The availability of soil water decreases progressively during terminal drought, and it may lead to severe drought stress at the later period of crop growth and development. Finite periods of inadequate rain or irrigation occurring at one or more intervals during the growing seasons is the condition of intermittent drought [27]. According to Crosser [28] drought delays formation of sugars, lowers energy exchange and destroys the entire biochemical processes. Heat stress at sowing directly affects crop germination and crop establishment. Chickpea seed germination decreases at supra-optimum temperatures [29]. Ellis *et al.* [30] indicated that the optimal temperature for germination is 10–15°C and noted that high germination temperatures are considered to be 22–35°C. The adaptive strategies to high temperature stress are classified into the following three groups [31].

2.1. Drought escape

Drought escape can be defined as the ability of a plant to complete its life cycle before a serious plant water deficit develops. Plants can escape heat stress with early phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water-deficit) and remobilization of pre-anthesis assimilates to grain [32]. Though flower initiation is sensitive to rising temperature in chickpea [33], early flowering and maturity is a heat escape mechanism [34] particularly in the Mediterranean spring-sown environments and south Indian germplasm. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape [35]. Crop duration is interactively determined by genotype and the environment and determines the ability of the crop to escape from climatic stresses including drought. Matching growth duration of plants to soil moisture availability is critical to realize high seed yield [36]. Drought escape occurs when phenological development is successfully matched with periods of soil

moisture availability, where the growing season is shorter and terminal drought stress predominates. In field-grown clones of Robusta coffee, leaf shedding in response to drought stress revealed that drought-sensitive clone has greater extent of leaf shedding [37]. Time of flowering is a major trait of a crop adaptation to the terminal drought and high temperature environments. Short-duration varieties to be developed to minimize yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress [38]. However, yield is generally correlated with the length of crop duration under favorable growing conditions, and any decline in crop duration below the optimum would tax yield [39].

2.2. Drought avoidance

The ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture; mechanisms for improving water uptake, storing in plant cell and reducing water loss confer drought avoidance is referred to as drought avoidance. Different mechanisms for drought avoidance are being reported in different plant species which include maintenance of turgor through increased rooting depth, efficient root system and increased hydraulic conductance and reduction in water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evaporation surface (leaf area). In crops, high root biomass has been of interest because the more the roots, the more their efficiency in absorption of water. This gives a plant more advantage in times when less moisture is available in the soil. A positive correlation between root system sizes and resistance to water stress has been found in several crops and many breeding attempts have focused on obtaining cultivars with larger root systems [40]. Saxena [41] has developed a chickpea cultivar with a greater degree of drought tolerance from combining large root traits of ICC4958. Similarly, Krishnamurthy et al. [42] has reported that large root biomass in a minicore collection of ICRISAT chickpea germplasm had high correlation with drought tolerance. Root system size is a complex trait since it is determined by intrinsic genetic factors and modulated by numerous environmental cues such as nutrient and moisture availability in the soil [43]. He also noted that smaller leaf surface was also a desirable trait related to drought tolerance. Plants with small leaf surface (pinnules) have shown to experience reduced water loss [41]. Glauconsness or waxy bloom on leaves helps with maintenance of high tissue water potential and is therefore considered as a desirable trait for drought tolerance [44, 45]. Varying percentage of glauconsness in wheat led to increased water-use efficiency, but it has minimal affect on total water use or harvest index. Determination of leaf temperature indicated glaucons leaves were 0.7°C cooler than non-glaucons leaves and had a lower rate of leaf senescence [43]. It was also suggested that a 0.5°C reduction in leaf temperature for 6 h per day was sufficient to extend the grain-filling period by more than three days. However, yield advantages are likely to be small as many varieties already show some degree of glauconsness.

2.3. Drought tolerance

The ability of plant to withstand water-deficit with low tissue water potential is referred as drought tolerance. A balance between maintenance of turgor and reduction in water loss helps plants to survive drought stress conditions [46]. Plants can combat drought stress by mainte-

nance of turgor through osmotic adjustment (a process which induces solute accumulation in cell), increase in elasticity in cell and decrease in cell size and desiccation tolerance by protoplasmic resistance [47]. Drought resistance is increased by maintaining plant turgor pressure. Drought tolerance characters studied are primarily involved with protection of cellular structure from the effect of cellular dehydration. Dehydrins and late-embryogenesis abundant (LEA) proteins are being accumulated in response to decrease in plant tissue water content [48]. These proteins are said to act as chaperones that protect protein and membrane structure [49]. Compatible solutes can also protect protein and membrane structure under dehydration [50]. The role of reactive oxygen species (ROS) in stress signaling have been extensively studied in recent years and reviewed [51, 52]. Consequently, crop adaptation must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity. Use of these traits as indirect selection for grain yield has been reported to be easier in breeding programs than selection based on direct grain yields [53].

2.4. Antioxidant defense

The antioxidant defense system in the plant cell constitutes both enzymatic and non-enzymatic components. Enzymatic components include superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase. Non-enzymatic components contain cysteine, reduced glutathione and ascorbic acid [54]. High activities of antioxidant enzymes and high contents of non-enzymatic constituents are important under drought stress conditions.

The reactive oxygen species in plants are removed by a variety of antioxidant enzymes and/or lipid-soluble and water soluble scavenging molecules [55] the antioxidant enzymes being the most efficient mechanisms against oxidative stress [56]. Along with catalase, various peroxidases and peroxiredoxins enzymes are involved in the ascorbate-glutathione cycle, this pathway allows the scavenging of superoxide radicals and H_2O_2 . Different enzymes that metabolize glutathione cycle are ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase [57]. Most of the glutathione cycle enzymes are found in the cytosol, stroma of chloroplasts, mitochondria and peroxisomes. Ascorbate peroxidase is a key antioxidant enzyme in plants whilst glutathione reductase has a central role in maintaining the reduced glutathione pool during stress [58]. Two glutathione reductase complementary deoxyribonucleic acids have been isolated; one type encoding the cytosolic isoforms and the other encoding glutathione reductase proteins dual-targeted to both chloroplasts and mitochondria in different plants [59].

Superoxide dismutase plays an important role, it catalyzes the dissociation of two molecules of superoxide into O_2 and H_2O_2 . Lima et al. [60] proposed that drought tolerance of a particular plant species can be associated with enhanced activity of antioxidant enzymes. In contrast, Pinheiro et al. [61] in his studies on four clones of *Coffea canephora* did not find a link between protection against oxidative stress and drought tolerance. Oxidative damage in the plant tissue is alleviated by both enzymatic and non-enzymatic antioxidant systems. These include β -carotenes, ascorbic acid, α -tocopherol, reduced glutathione and enzymes including superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase and glutathione reductase [62, 63]. Carotenes are crucial part of the plant antioxidant defense system [64]; in

spite of this, they are very susceptible to oxidative destruction. The β -carotene present in the chloroplasts of all green plants is exclusively bound to the core complexes of photosystem I and photosystem II. Protection against damaging effects of reactive oxygen species at this site is essential for chloroplast functioning. β -carotene functions as an accessory pigment, it also acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them. β -carotene also has a protective role in photosynthetic tissue by direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage.

2.5. Plant growth regulators

Plant growth regulators phytohormones are substances that influence physiological processes of plants at very low concentrations, either they are applied externally or produced in the plant [65]. Both these terms have been used interchangeably, particularly when referring to auxins, gibberellins, cytokinins, ethylene and abscisic acid [66]. Under drought, endogenous contents of auxins, gibberellins and cytokinin usually decrease, while those of abscisic acid and ethylene increase [67]. Nevertheless, phytohormones play vital roles in drought tolerance of plants. Auxins break root apical dominance helping in new root formation induced by cytokinins. Drought stress limits the production of endogenous auxins, usually when contents of abscisic acid and ethylene increase. Application of indole-3-yl-acetic acid exogenously enhanced net photosynthesis and stomatal conductance in cotton (Kumar et al., 2001). Indole-3-butyric acid is a naturally occurring auxin. Enhanced indole-3-butyric acid synthesis was observed in maize in response to drought stress and abscisic acid application. Enzyme indole-3-butyric acid synthetase was revealed from *Arabidopsis* under drought stress [68]. Experiments with indole-3-yl-acetic acid and ethylene glycol tetra-acetic acid suggested that calcium and auxin participate in signaling mechanisms of drought-induced proline accumulation [69]. An adaptive strategy that occurs during progressive drought stress is drought rhizogenesis. Families such as Brassicaceae form short and tuberized, hairless roots in response to drought stress. These roots are capable of withstanding a prolonged drought period and give rise to a new functional root system upon rehydration. The drought rhizogenesis was highly increased in the gibberrellic acid biosynthetic mutant *ga5*, suggested that gibberrellic acids also participate in this process [70]. Abscisic acid is a growth inhibitor and produced under a wide variety of environmental stresses. All plants respond to drought and many other stresses by accumulating abscisic acid. Abscisic acid is ubiquitous in all flowering plants and is generally recognized as a stress hormone that regulates gene expression and acts as a signal for the initiation of processes involved in adaptation to drought and other environmental stresses. It has been proposed that abscisic acid and cytokinin have opposite roles in drought stress. Increase in abscisic acid and decline in cytokinins levels favor stomatal closure and limit water loss through transpiration under water stress [71]. Increased abscisic acid concentration leads to many changes in development, physiology and growth. Abscisic acid alters the relative growth rates of various plant parts such as increase in the root-to-shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots. It triggers the occurrence of a complex series of events leading to stomatal closure, which is an important water conservation response [72]. In a study on genetic variation for abscisic acid accumulation in rice, a

consistent negative relationship between the ability of detached and partially dehydrated leaves to accumulate abscisic acid and leaf weight was established [73]. By its effect in closing stomata, abscisic acid can control the rate of transpiration and, to some extent, may be involved in the mechanism conferring drought tolerance in plants.

Ethylene is considered as growth inhibitory hormone, it is involved in environmentally driven growth inhibition and stimulation [66]. The response of cereals to drought includes loss of leaf function and premature onset of senescence in older leaves. Ethylene regulates leaf performance throughout its lifespan as well as to determine the onset of natural senescence and mediate drought-induced senescence [74]. Recent studies suggest that growth promotion is a common feature in ethylene responses. To escape this adversity, plants can optimize growth and tolerate abiotic stresses such as drought, and this response also involves ethylene synthesis [75].

Polyamines are known to have profound influence on plant growth and development. Being cationic, polyamines can associate with anionic components of the membrane, such as phospholipids, thereby protecting the lipid bilayer from deteriorating effects of stress. There has been a growing interest in the study of polyamine participation in the defense reaction of plants against environmental stresses and extensive research efforts have been made in the last two decades [76, 77]. Different genes for enzymes involved in polyamine metabolism has been analyzed for their expression under drought stress in several species. For example, the apple spermidine synthase gene when overexpressed encodes high levels of spermidine synthase, which substantially improves abiotic stress tolerance including drought [78].

3. Morphophysiological mechanisms for drought stress in plants

Water limitation is one of the important factors limiting crop productivity worldwide. Nearly all terrestrial plants are exposed to drought stress at different times and to different intensities during their life cycle [79, 80]. As water is fundamental to almost all aspects of plant growth, plants are thought to have evolved numerous strategies for coping with limited water availability including changes in phenological developmental and physiological traits [81, 82].

3.1. Phenological traits

3.1.1. *Early flowering and maturity*

Early maturity is an important trait to avoid drought stress. Early flowering and early podding are two main components of drought escape in crops to avoid higher yield losses from drought. The differential genotypic response to drought stress, as a result of variation in physiological parameters has also been reported by Gunes et al. [83]. Early maturing chickpea varieties that escape terminal drought have been developed, but early maturity decreases yield and limits the crop's ability for extended growing periods. Chickpea genotypes with high growth vigor showed early maturity. Selection for high growth vigor enhances chances for escaping terminal drought stress [84]. Initial growth vigor is suitable character for large-scale evaluation of germplasm and breeding materials [85].

3.1.2. *Root and shoot traits*

Extensive and deep root systems have been recognized as one of the most important traits for improving crop productivity under progressively receding soil moisture condition. Roots have a major role in dehydration avoidance as deep root system is able to obtain moisture from the deeper soil layers even when the upper soil layer becomes dry. The root traits such as biomass, length density and depths have been proposed as the main drought avoidance traits to contribute to seed yield under terminal drought environment [83]. Upadhyaya et al. [86] observed chickpea variety ICC13124 was equally good in respect of root traits (root length, root weight and root volume) as compared to ICC4958. Shoot fresh weights were significantly greater in well watered genotypes, but there was no significant effect of moisture stress on shoot dry matter content, revealing that weight of fresh shoot was higher due to high uptake of water under well watered conditions which evaporated after drying.

3.2. **Physiological traits**

3.2.1. *Leaf water status*

Moisture deficit affects plant establishment in the field, photosynthetic ability and osmotic behavior of cells. However, species and genotypes vary in their capacity to tolerate water stress [87]. Plants adopt various defense mechanisms in response to terminal drought which are accomplished by regulating internal plant water status. Plant water status that includes leaf water potential, osmotic potential and relative water content represents an easy measure of water deficit and provides best sensor for stress. Water stress reduces the osmotic potential of tissues in the plant which helps in maintenance of turgor potential for normal metabolic activities which has been recognized as basic mechanism of drought tolerance [88]. Gupta et al. [89] studied the physiological mechanism of drought tolerance in chickpea. It was observed that tolerant genotype had lower membrane injury, retain imbibitions seedling growth, osmotic adjustment and water use efficiency. A partial closer of stomata led to decreased conductance under water stress resulting into reduced transpiration and photosynthesis has been reported by (Sharma and Singh) [90]. Kushwaha et al. [91] indicated that genotypes which possessed high initial water content (IWC) along with high relative water content resulted in relatively less damage to the assimilatory system resulted in to the production of relatively higher biomass. The osmo-regulatory activities helped the plant to cope up with moisture stress. Variation in RWC is achieved through differences in plant ability to absorb water from soil by developing a high water potential gradient from soil to plant, extending rooting depth or ability to control water loss through stomata [92]. A decrease in the relative water content (RWC) in response to drought stress has been recorded in wide variety of plants as reported by Nayyar and Gupta [93].

3.2.2. *Relative stress injury, CTD and photochemical efficiency*

The role of cell membrane remains to be more critical for adaptation under temperature and moisture stress conditions. Blum and Ebercon [94] described that under water stress conditions measurement of electrolyte leakage can be used to estimate water stress tolerance. Heat tolerant

genotypes were able to possess higher membrane stability [95]. Higher membrane stability in drought tolerant genotypes under stress was due to increased activities of antioxidative enzymes which prevent damage of membrane by active oxygen species produced under stress. It had been reported that tolerant and intermediate genotypes were superior to susceptible ones in maintaining membrane stability and lower membrane injury under drought stress condition [96]. Stomatal closure occurs when plants are subjected to water stress in order to decrease energy dissipation. Transpiration plays a major role in leaf cooling and reduces canopy temperature relative to ambient temperature. Relatively lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status. The photosynthetic efficiency, transpiration and the values of relative stress injury declined in chickpea under drought conditions [97]. Photosynthetic pigments play an important role in light harvesting and dissipation of excess energy. It is known that the content of both chlorophyll a and b changes under drought stress [98]. Carotenoids participate in energy dissipation and can aid plant resistance against drought stress.

4. Breeding for drought tolerance

Drought offers great challenges to plant breeders around the globe. Drought is usually uncertain and unpredictable in the field and response of canopy toward drought is perceived using conventional techniques mainly. Conventional breeding procedures such as introduction, selection, hybridization and mutation are widely used by breeders. In spite of conventional methods novel methods such as *in situ* and *in vitro* techniques can also be used for selection, survival rate or to monitor gene expression changes of wild-type plants genotypes overexpressing candidate genes for drought tolerance. Plant responses to drought at both the physiological and molecular levels are studied extensively. Major drawback of studies for drought treatments is uncontrolled soil water moisture and comparison of performance of different genotypes with different growth characteristics. In environment, drought often develops during a growing season and occurs for a short period, which tolerant plants can manage to survive and complete their growth cycle. Drought resistance mechanisms can be understood by methods which simulate field-like conditions and quantify drought responses. Soil water deficit causing drought stress in crop plants has been tested in *Arabidopsis* using controlled soil moisture treatment. Controlled drought treatment, exposing plants to constant levels of soil moisture deficit, enables the evaluation between genotypes/ecotypes for plant responses to sublethal drought. Phenopsis is an alternative method for an automated controlled drought screen, which is used to compare the performance of different *Arabidopsis* ecotypes (accessions) and resulted in the identification of a resistant accession, An1 [99]. Controlled drought was also used to study the response of the *Arabidopsis erecta* mutant and *ERECTA* gene complementation [100], the overexpression of the *Arabidopsis* *ESKIMO1* gene [101] and overexpression of the *Pro* biosynthesis gene in chickpea [102]. Comprehensive physiological and molecular studies have not yet been done on the response of plants to moderate drought (mDr). A transcriptome study in loblolly pine (*Pinus taeda*), treated for

cycles of mild drought and recovery [103], revealed a photosynthetic acclimation pattern in response to mild drought in contrast to photosynthesis inhibition under severe drought. A comprehensive understanding of the response of plants to mDr with physiological and molecular tools provided a better understanding of the acclimation process. A semi-automated, controlled mDr testing system was employed to compare with pDr treatment for physiological and molecular responses. This revealed differential gene reprogramming under the two drought treatments. The dissection of mDr treatment is presented using a time-course study to provide a picture of physiological and molecular responses toward acclimation in plant growth.

In recent years, much molecular information has been generated on the response of plants to environmental stresses. Plants respond to environmental stresses such as drought by the induction of both regulatory and functional sets of genes. Very little is known about the early events in the perception of stress signals. The common stress signaling pathways have been distinguished into abscisic acid (ABA) dependent and ABA independent. Most of the key genes in these pathways have been identified, such as transcription factors belonging to the class of DRE-binding protein (DREB)/C-repeat-binding factor (CBF), ABA-binding factor (ABF), MYC and MYB, including the identification of the stress-responsive cis-elements ABA-responsive element (ABRE) and dehydration responsive element. Downstream of the early signal perception events, signaling genes and molecules acting as secondary messengers have been identified, revealing the role of Ca^{2+} and reactive oxygen species (ROS) as secondary messengers. These regulatory mechanisms induce downstream functional genes, which are needed to establish new cellular homeostasis that leads to drought tolerance and/or resistance. Most of our knowledge of drought responses at the molecular level is based on plant responses to molecular laboratory experimental conditions of dehydration and/or osmotic treatments. Laboratory conditions are far from the soil water deficit met by plants under field conditions, but these studies have provided valuable knowledge. Signaling pathways of ABA dependent and ABA independent have become a paradigm in plant biotic/abiotic stress responses [104]. These pathways were discovered in *Arabidopsis* (*Arabidopsis thaliana*) as a model system, which paved the way for the discovery of parallel pathways in other crop plants such as in rice (*Oryza sativa*) as a model for monocot plants. A number of drought treatments have been used to test the response of plants for improved tolerance/resistance. One method is progressive drought (pDr), in which water is withheld for a certain period of time until symptoms of wilting are observed. Usually, this method of drought treatment has been used to determine survival rate or to monitor gene expression changes of wild-type plants or of plant genotypes overexpressing candidate genes for drought tolerance. These studies have helped to study plant responses to drought at both the physiological and molecular levels. However, one of the drawbacks of pDr treatment is that it cannot be used to compare the performance of different genotypes with different growth characteristics. In nature, drought often develops during a growing season and occurs for a short period, which tolerant plants can manage to survive and complete their growth cycle. Soil water deficit causing drought stress in crop plants has been tested in *Arabidopsis* using controlled soil moisture treatment. Controlled drought treatment, exposing plants to constant levels of soil moisture deficit, enables the evaluation between genotypes/ecotypes for plant responses to drought.

A large variety of stress responses in plants are influenced by ethylene metabolism and signaling. Ethylene signaling pathway is a major cross-link between ethylene and other plant hormones metabolism (ABA and GA). Interactions between ethylene and other plant hormones also benefit immediate stress responses such as stomatal closure as well as long term adaptations under severe drought conditions. Candidate genes that are related to maintenance of growth under low water conditions are agronomically important since they provide an efficient resource for crop improvement. A transgenic potato (*Solanum tuberosum*) cultivar, containing a betaine aldehyde dehydrogenase (*BADH*) gene from spinach (*Spinacia oleracea*) under the control of a stress induced *Arabidopsis* promoter, has been reported to exhibit improved growth after induction of *BADH* by NaCl and drought stress.

Proline an osmolyte in plants accumulates under different stress conditions. The amino acid proline in plant cells contribute to osmotic adjustments under adverse conditions. The enzyme Δ 1-pyrroline-5-carboxylate synthetase (*P5CS1*) is a major component in proline biosynthesis. A study with *P5CS1*-deficient *Arabidopsis* mutants indicated that proline synthesis is required in order to maintain growth at low water availability [105]. Proline dehydrogenase 1 (*PDH1*)-deficient *Arabidopsis* mutants with blocked proline catabolism exhibited decreased root growth, fresh weight and dry weight. Additional components of the proline biosynthetic pathway are associated with stress responses. In transgenic soybean (*Glycine max*) with a Δ 1-pyrroline-5-carboxylate reductase (*P5CR*) gene and the antisense construct from *Arabidopsis*, it was found that proline might enhance survival during drought stress [106]. The *P5CR* gene and antisense construct were manipulated using an inducible heat shock promoter (*IHSP*). Two transgenic potato lines, which expressed a trehalose-6-phosphate synthase (*TPS1*) gene from yeast (*Saccharomyces cerevisiae*) were found to be more effective in keeping water and acceptable levels of photosynthesis during drought compared to WT-plants [107]. The expression of aldehyde dehydrogenases (*ALDHs*) is upregulated under stress situations such as dehydration, salinity and oxidative stress. *ALDHs* are able to convert highly reactive aldehydes and hence extenuate oxidative stress [108]. Two drought tolerant Andean native potato clones (*Solanum tuberosum* subsp. *andigena*) under transcriptome analysis showed that aldehyde dehydrogenase family 7 (*ALDH7*) was induced under drought stress conditions [109]. Functional analyses of an *ALDH7* gene member (*GmPP55*) from soybean (*Glycine max*) confirmed these studies. Transgenic *Arabidopsis* and tobacco (*Nicotiana tabacum*) plants exhibited improved tolerance to H_2O_2 as well as salt and drought conditions during different developmental stages [110].

5. MAS for drought tolerance

Conventional breeding for developing drought-tolerant crop varieties is time-consuming and labor intensive due to the quantitative nature of drought tolerance and difficulties in selection for drought tolerance. The identification of genomic regions associated with drought tolerance would enable breeders to develop improved cultivars with increased drought tolerance using marker-assisted selection (MAS). Plant breeding has benefited from DNA marker technologies that were used to establish saturated genetic maps in major crop species including cereals and

legumes. Markers in a high density genetic map will allow the precise tagging of mono- and oligogenic traits, with the dual goal of marker-assisted selection for traits and positional cloning of the underlying genes. Use of genomic tools like molecular markers and other tools in integrated approach for crop improvement has also been referred as “genomics- assisted breeding”. Mapping of genomes has been of interest to identify genomic locations of disease resistance genes and other yield-related traits. However, due to very low polymorphisms in few cultivated crops gene pool, progress in genomic research has been relatively slow compared with other highly polymorphic species. Important considerations for undertaking molecular breeding are molecular markers, genetic maps and markers associated with traits. During the early days of genomic studies, isozyme markers were used for map development in chickpea. Expression of these markers was influenced by the environment and their number was small. Restriction fragment length polymorphism (RFLP) and Random amplified polymorphic DNA (RAPD) markers were also used for genetic mapping studies. After development of simple sequence repeat (SSR) or microsatellite markers, the use of molecular markers increased extensively. SSR markers were considered as the marker of choice in plant breeding due to their multi-allelic and co-dominant nature. Several hundred SSR markers have been developed from genomic DNA libraries. In several cases, the mapping populations used for developing the maps were also phenotyped for the segregating traits. Analysis of phenotyping data together with genotyping data in some cases identified molecular markers associated with the genes/quantitative trait loci (QTLs), controlling resistance to key diseases (ascochyta blight, fusarium wilt, botrytis grey mold, rust), morphological traits (single pod *vs.* double pod, flowering time and flower color), seed yield and yield components, *etc.* Marker-assisted breeding reduces the effect of environmental conditions during the selection process, which is a major hindrance in conventional breeding under drought.

5.1. QTL analysis for drought tolerance in chickpea

Compared to the conventional breeding approaches for improved productivity under water limited environments, genomics offers great opportunities for dissecting quantitative traits into their single genetic determinants. The release of varieties through conventional breeding approaches is coupled with identification of several large-effect QTLs for grain yield under drought in different crops. Independent and epistatic QTLs for grain yield and other traits of agronomic importance were studied in different crops. Only a few studies reported major QTLs affecting yield advantage under both drought stress and non-stress environments. Drought is normally associated with increased incidence of diseases such as blast, brown spot, and bacterial blight. Few studies have been undertaken to understand the genetics of these abiotic and biotic stresses simultaneously in a mapping population. Identification of QTLs is paving the way to MAS and assisted pyramiding of the beneficial QTL alleles. Markers can be used in marker-assisted selection (MAS) for improving the desired trait. Isolation and validation of genes underlying the QTL/genes for the traits of interest is an essential step to determine gene function. QTLs for drought tolerance have been identified for several major and important crop species such as rice, maize, wheat, barley, sorghum, pearl millet, soybean and chickpea. These QTLs were identified for a variety of important traits including: (1) yield and yield-contributing traits under water-deficit conditions (in the case of wheat, maize, rice, soybean

and pearl millet), (2) physiological responses including water-soluble carbohydrates, carbon isotope ratio, osmotic potential, chlorophyll content, flag leaf rolling index, grain carbon isotope discrimination, relative water content, leaf osmotic potential, osmotic adjustment, chlorophyll and chlorophyll fluorescence parameters to drought stress (in the case of wheat, maize and rice), (3) flowering time including anthesis to silking interval (in maize), (4) root traits (rice, maize, wheat, soybean and chickpea), (5) stay green (sorghum) and (6) nitrogen fixation (soybean). When the QTLs identified for drought tolerance traits contribute higher phenotypic variation, they are considered major QTLs. These QTLs, after validation in desired germplasm, can be used for introgressing drought tolerance from the donor genotypes (generally used for identification of the QTL for the trait) into elite, less drought-tolerant cultivars or breeding lines (recipient parents) without transfer of undesirable or deleterious genes from the donors (linkage drag). After identifying important QTLs, the next step involves the identification of candidate sequences, validate their role and proceed with the direct manipulation using the gene itself as marker for MAS. In chickpea the RILs of ICC 4958 × Annigeri have been extensively studied for root traits. An SSR marker (TAA 170) was identified for a major QTL that accounted for 33% of the variation for root weight and 33% of the variation for root length [111]. Recent preliminary screening of the chickpea mini-core germplasm collection for root proliferation and depth in cylinder culture indicated that contrasting parents are available with wider variation for these traits than that present between ICC 4958 and Annigeri [112]. Nayak et al. [113] undertook identification of QTLs and genes for drought tolerance using linkage mapping and association mapping approaches in Chickpea (*Cicer arietinum*). SSR markers were tested for polymorphism on parental genotypes of the inter-specific (ICC 4958 × PI 489777) and intra-specific mapping population (ICC 4958 × ICC 1882). As a result, a comprehensive inter-specific genetic map of 621 marker loci, spanning a genetic distance of 984.11cM was prepared. Varshney et al. [114] identified genomics and physiological approaches for root trait breeding to improve drought tolerance in Chickpea (*Cicer arietinum* L.). Molecular markers and candidate genes associated with root traits are being targeted to introgress the QTLs for root traits from drought-tolerant genotypes to drought-sensitive genotypes following marker-assisted breeding strategies. Varshney et al. (2014) reported a “QTL-hotspot” (ICCM0249, NCPGR127, TAA170, NCPGR21, TR11, GA24 and STMS11) on CaLG04 in the chickpea genome, identified in analysis on both RIL populations, (ICCRIL03 (ICC 4958 × ICC1882) and ICCRIL04 (ICC 283 × ICC 8261) that contain 45 M-QTLs and 973 E-QTLs for several drought tolerance traits contributing up to 58.20% phenotypic variation for targeted traits [22, 115].

In the last 20 years, considerable progress has been made towards mapping QTLs for drought resistance traits in rice however, there have been few successful cases of their application in MAB. The success rate of using QTLs in molecular breeding reflects the lack of repeatability of QTL effects across genetic backgrounds and environments. In recent years, several researchers developed mapping populations between high-yielding lines (IR64, Swarna and MTU1010) and drought-tolerant local landraces and wild cultivars to map grain yield QTLs for reproductive stage-specific drought stress.

To the best of our knowledge, none of the studies were conducted under natural drought conditions predominant in tough environments (TEs) and these QTLs were identified in moderate stress environment (MSE) and QTLs mapped under severe drought stress conditions. Successful marker-assisted selection to improve yield mainly relied on the use of high yielding lines to identify large-effect QTLs and evaluation of their consistent effects. Studies in MSE may limit the chances of detecting QTLs for drought resistance that are widely applicable to target populations of environments, as the timing and intensity of stress vary over years in rain fed rice ecosystems, which ultimately changes the plants' responses and traits involved in drought-resistance mechanisms. Most of the indica × indica derived rice lines used in QTL mapping of drought resistance were not adapted to TEs. The importance of field experiments in TPEs to identify QTLs for rice yield under natural drought stress was emphasized. Recombinant inbred lines (RILs) derived from locally adapted indica rice lines to detect QTLs for plant production traits under drought stress in TPEs, but no yield QTL was identified.

Quantitative genetics, with wide range of molecular markers available, provide identification of the genetic factors (quantitative trait loci-QTLs) responsible for expression of traits. Recent development in molecular marker technology is expected to enable greater power in detection of QTL for agronomically important traits and utilization of QTL information for crop improvement. Thus marker-assisted selection could significantly enhance in improving crop drought tolerance, if QTL with significant effects can be identified.

6. Conclusion

Environment change is a universal phenomenon that has started to have adverse impact on agriculture. The global temperature is predicted to rise by 2.5–4.3°C by the end of the century. The situation is further likely to get worse due to the occurrence of increase in the irregularity of rainfall, drought, flood and land degradation. With predicted climate change scenarios and continuous population explosion, there is a great need to develop high-yielding varieties with improved drought tolerance. Breeding for drought tolerance is not simple. Under a particular environment, some physiological or metabolic processes can be modified through breeding, either as single traits or as a combination of traits. Optimal drought-adaptation requires the combination of several morphological, physiological and phenological processes which depends on multiple genes and varies within each target environment. Conventional and marker-based approaches coupled with each other have been used for drought tolerance. The conventional breeding approach is based on selection for yield and its components in a given drought environment. But this approach requires large investments in land, labor and capital to screen a large number of progenies plus the difficulty of sampling even a part of the expected range of variability in stress occurrence in the target environment hence this approach is not successful. Traits including modification of the root system, stomatal control, and leaf area, as well as matching plant phenology with the environment, could help in improving productivity under drought stress conditions. Recent research breakthroughs in biotechnology have revived interest in targeted drought tolerance breeding and use of new genomics tools to increase crop productivity.

Marker-assisted breeding is an important technique for improvement of crop productivity against drought stress. As a complement to the recent rapid progress in genomics, a better understanding of physiological mechanisms of drought response contributes to the progress of crop productivity against drought tolerance. Mostly physiological traits associated with drought tolerance are quantitative in nature. An important research strategy that has been widely used over the past two decades to deal with such complexity is the use of molecular markers to identify quantitative trait loci (QTLs) in appropriate mapping populations. Once molecular markers (i.e. for trait QTLs) linked to specific drought tolerance component traits found, it is possible to move them into adapted cultivars or other agronomic backgrounds through marker-assisted breeding. Moreover, in adapted genotypes identification of QTLs for the key traits responsible for improved productivity under drought could be helpful in accelerating the process of pyramiding of favorable alleles for better yield and production. Integration of knowledge from plant physiology and biotechnology into plant breeding can help developing best cultivars for drought tolerance. The availability of a large number of molecular markers, dense genetic maps, and markers associated with traits and transcriptomics resources have made it possible to integrate genomics technologies into chickpea improvement. Understanding plant response to water stress for key drought stress traits and screening of mapping populations for these traits for QTL identification are of prime importance for future drought stress breeding. Food security requires investments in this domain, in particular with new genotypes that can at least maintain an acceptable productivity under drought stress condition.

Author details

Summy Yadav¹ and Kamal Dutt Sharma^{2*}

*Address all correspondence to: kdutt1966@gmail.com

1 Ahmedabad University, Gujarat, India

2 CCS Haryana Agriculture University, Haryana, India

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