Wheat (Triticum aestivum L.): A drought condition morphological, biochemical and molecular effect on vegetative and reproductive stage

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Abstract
Wheat (Triticum aestivum L.) is the most important cereal crop for the majority of world’s populations and staple food of about two billion people. Wheat is cultivated over a wide range of climatic conditions and wheat are exposed to numerous biotic and abiotic stresses which is of a significant effect on the growth, development and cause changes in the normal morphological, physiological, and molecular functions of the plants. Drought stresses are significant causes of crop loss. Drought stresses are often interconnected, and could induce similar cellular damage, plants must adapt to stressful conditions, and exercise specific tolerance mechanisms modification for enhanced tolerance is mostly based on the manipulation of genes that maintained and protect the structure, and function of cellular components. Present strategies rely on the transfer of one, or several genes that are either involved in signalling and regulatory pathways, or that encode enzymes present in pathways leading to the synthesis of functional and structural proteins or that encode stress-tolerance conferring proteins. Tolerance to abiotic stresses be a very complex due to intricate interactions between stress factors and various molecular, biochemical, and physiological phenomenon affecting plant growth and development.

Keywords: Drought, morphological, biochemical and molecular response, drought tolerance

Introduction
Wheat (Triticum aestivum L.) is world’s most widely cultivated food crop in India and second important staple cereal food. Wheat is consumed in various other forms such as chapatties, poories, dalia, halwa, sweet meal and contains more nutrient protein [8-15% (grain), 8-13% (flour)] and71% carbohydrates, iron, fibre, vitamin and calories which almost 72% in average diet than other cereals. Proteins are of special significance role in nutrition; these are principally concerned with providing the characteristic substance gluten which is very essential for backers. Grown over 200 mha in the range of environment throughout the world with an annual production (Reynolds et al., 2008) [51]. Current production is not sufficient to meet the demands of rapidly growing population. (Moaveni 2011). Although breeders are working hard to improve wheat production, however increasing wheat production in drought environments has been more to achieve (Jatoi et al., 2011) [28].

Taxonomic status and genome
Belongs to family Gramineae (Poaceae) which includes major crop plants such as wheat (Triticum spp.), barley (Hordeum vulgare L.), oat (Avena sativa L.), maize (Zea mays L.) and rice (Oryza sativa L.). Triticeae is one of the tribes containing more than 15 genera and 300 species including wheat and barley. Linnaeus in 1753 first classified wheat. In 1918, Sakamura reported the chromosome number sets for each commonly recognized type. He separated wheat into three groups viz. Diploids (2n=14), tetraploids (2n=28) and hexaploids (2n=42) chromosomes. The wheat evolution of hexaploid of Triticum aestivum L. (AABBDD) is well known as the tetraploid wheat (Triticum turgidum L.) (AABB) first originated from the species of A genome (Triticumurartu tumanian ex Gandilyan) (Dvorak et al., 1993) [17] and B genome ancestor Aegilops speltoides Tausch (SS) and then tetraploid wheay hybridizing under natural condition with D genome donor i.e. Aegilopstaus chii Coss.
The wheat diversity available in D genome of cultivated hexaploid wheat is very limited in comparison to available in D genome of diploid species. (Dvorak et al., 1998) [16]. It has one of the largest and complex genomes of cereals. It is allohexaploid (2n=6x=42, AABBDD) with three homologous genomes (Sears, 1954) [57]. Haploid genome of hexaploid wheat is 16 Mb (16 billion base pairs of DNA). Genetic diversity is the basis for the improvement of traits (Huang et al., 2002) [22].

**Fig 1:** Diagram shows the natural evolution of the two most common hexaploid wheat, spelt and bread wheat. The top, and the right side of the diagram shows the evolution of spelt and bread wheat. On the left side of the diagram durum wheat, a common tetraploid wheat and diploid goat grass are shown.

### Drought stress

In arid and semi-arid regions with Mediterranean climate wheat crops usually encounter drought during the grain filling period. Drought is the most damaging abiotic stresses affecting agricultural crops. It is abiotic stress affecting the yield, and yield stability of food cereals and acts simultaneously on many traits leading to a decrease in yield. Plants are exposed to a wide range of environmental factor during their life of Plant. Drought stresses lead to physiological and developmental changes, decrease of soil water potential so plants reduce their osmotic potential for water absorption by conge stion of soluble carbohydrates, proline and other words osmotic regulation is performed (Martin, et al., 1993) [42]. Therefore, osmotic regulation will help to cell development and growth of plant in drought stress (Pessarkli, 1999) [49]. It is defined that decrease of relative water content (RWC) closed stomata and also after blocking of stomata of cell will reduce photosynthesis rate (Cornic, 2000; Sangtarash, 2010) [15, 54].

### Drought stress of wheat

Drought stress adversely affects wheat production, quantity and quality in many regions of the world, the loss of which is the total for other natural disasters, with increasing global climate change making the situation more serious (Kirigwi et al., 2007; Turner, 1991) [31, 65]. Drought avoidance can be defined as the plant’s ability to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress (Levitt, 1980) [35]. It identified two plant types with respect to dehydration, avoidance i.e. ‘water savers’ and ‘water spenders.’ Important features of these plants are root characteristics (increased water uptake), leaf and stomatal characteristics (reduced water loss) and osmotic adjustment to lower the osmotic potential (Blum, 1988; Acevedo and Fereres, 1993) [11, 1]. Grain yield is a product of an organized interplay of several factors, which are highly susceptible to environmental fluctuations. However, grain yield can be estimated based on performance of various yield traits. Yield is a complex character dependent upon the interaction of environment and genetic of the wheat plant. Apart from direct selection for grain yield, enhancement in most situations is more effectively fulfilled based on performance of yield components, which are closely associated with yield (Ashfaq et al., 2003) [7]. Correlation coefficients between different parameter with the grain yield to decisions about the relative importance of these attributes, and their values as selection criteria helps. According to several reports, between grain yield, and grain weight, fertile tillers or spikes per plant, spikelet’s per spike and spikelet weight have a significant correlation (Shanahan et al., 1985; Khayatnezhad et al., 2010).
Path analysis show direct and indirect effects of course variables on effect variables. In this method, the correlation coefficient between two traits is separated into the components which measure the direct and indirect effects of stress condition (Ahmadizadeh et al., 2011) [3].

**Morphological responses under drought condition**

At the cellular level plant responses to water deficit may result in cell damage, whereas other responses may correspond to adaptive processes. Although many drought induced genes have been identified in a wide range of plant a molecular basis for plant tolerance to drought stress remains far from being completely understood (Ingram and Bartois, 1996) [24]. Therefore, it is difficult to determine precise relationship between plant physiological responses to drought induced gene (Cellier, 1998) [14] drought stress is characterized by reduction of water content, diminished leaf water potential, turgor loss, closure of stomata, decrease in cell enlargement and growth (Jaleel et al., 2007) [26]. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plants (Jaleel et al., 2008) [27]. Plant growth is accomplished through cell division, cell enlargement and differentiation, which involve genetic, physiological, ecological, and morphological events; sensitive to drought (Taiz and Zeige, 2006; Farooq et al., 2009). Water shortage at a critical growth stages such as crown root initiation, tillering, booting, anthesis and grain filling stage has deleterious effects on plant growth, development and yield (Khan, 2003; Manikavelu et al., 2006; Edward and Wright, 2008).

The first response of virtually all plants to acute water shortage is the closure of stomata to prevent transpiration water loss (Manisfield and Atinson, 1990), which may result in response to decrease in leaf turgor and water potential (Yokota et al., 2002). Drought hampers photosynthesis due to reduce synthesis of chlorophyll pigments resulting in a controlled light harvested reaction (Jaleel et al., 2009) [25]. The other reduction of synthesis in photosynthetic rate are decrease ins leaf expansion, impaired photosynthetic machinery, reduced influx of CO2 due to low stomatal conductance and premature leaf senescence (Wahid and Rasool, 2005) [68]. Drought stress lowers water potential, osmotic potential and pressure potential, mostly reduces leaf growth and in turn leaf area in many plant species (Farooq et al., 2009; Akram, 2003) [19, 4]. The best source for food production in spikes is the flag leaf. Higher flag leaf area is important to spike production as a large amount of grain yield per spike is affected by the area of the adjacent flag leaf. More leaf area particularly the flag leaf increase photosynthesis in the plant. Late season drought stress is one of the most important limiting factors. (Akkari et al., 2002) [3] determined that omitting water at spike production stage had the most effect on reducing grain yield (about 36%) and total yield (about 20%). Wheat crop needs water for whole growth period, but some stages are more vulnerable to water shortage of irrigation water at crown root initiation, booting, and early grain fill period results in significant yield losses (Anonymous, 2007) [6].

**Physiological responses under drought condition**

Drought stress tolerance is seen in almost all plant species but its extent varies from species to species and even within species due to differences morphological, biochemical, physiological, and molecular adaptive mechanisms (Nakayama et al., 2007) [45]. Drought stress tolerance describes the ability of plants to continue metabolizing at low leaf water potential and to maintain growth dehydration of the tissue. According to (Hsiao, 1973 and Boyer, 1976) [11, 12] translocation is one of the more drought tolerant processes in plants. It would proceed at levels of dehydration sufficient to inhibit photosynthesis. When drought stress occurs and the current photosynthetic source is inhibited, the role of stem reserves may therefore be considered as a powerful resource for grain filling in stress-affected plants during the grain filling stage and associated with various physiological factors such as levels of a certain stress hormone viz. ABA. Which regulates stomata conductance and hence water loss under desiccation (Kriedemann et al., 1972) [33] and accumulation of osmolites like proline, manitol, glycine betaine and soluble sugars which lower the osmotic potential of the cell sap thus prevent the movement of water out of cell (Stewart et al., 1855) [59].

**Relative Water Content (RWC)**

The RWC of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity. RWC provides a measurement of the water deficit of the leaf, and may indicate a degree of stress expressed under drought condition. RWC integrates leaf water potential with the effect of osmotic adjustment (a powerful mechanism of conserving cellular hydration) as a measurement of plant water status. A genotype with the ability to minimize stress by maintaining turgid leaves in stressed environments will have physiological advantages. RWC is easily and simply measured, without the need for expensive specialized instruments. Fresh leaf samples to field grown crops are first weighed then placed in water, chilled overnight, and re-weighed before being oven dried and weighed a final time. The RWC difference in leaf samples provides a quantitative measure of their infiel hydration status. Trials can be rapidly screened for genotypes which maintain high leaf RWC values during water deficit stress and vice-versa. Sources of error in the estimation of Relative Water Content can be summarized as:

a. Change in dry weight (mainly due to respiratory losses),

b. Increases in water content in excess of full turgidity, and

c. Water accumulation in intercellular spaces (Barrs and Weatherley, 1962).

RWC is closely related with cell volume, it may more closely reflect the balance between water supply to the leaf and transpiration rate (Schonfeld, et al., 1998). This influences the ability of the plant to recover from stress and consequently affects yield stability (Ludlow, 1996). In mid 80s, RWC was introduced as the best criterion for plant water status which, afterwards was used instead of plant water potential as relative water content referring to its relation cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration. (Schonfeld et al., 1988) [56] Showed that wheat cultivars having high relative water content are more tolerant against drought stress. Generally, it seems that osmoregulation is one of the main mechanisms preserves turgor pressure in most planting species against water loss from so, it causes plant to continue water absorption and retain metabolic activities and indicates that water status of the cells and has significant association with yield and stress tolerant. The difference in RWC in wheat leaves may also be due to different in the ability of the tested variety to accumulate (Almeselmani et al., 2012) [5]. RWC is useful means for determination the physiological
water status and indicates degree of drought stress of plant (Makbul et al., 2011).

Chlorophyll content
Chlorophyll content is one of the major factors affects photosynthetic capacity in plant. Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plants species and its intensity depends on stress rate and duration (Rensburg and Kruger, 1994; Nageswara et al., 2001) [50, 44]. Flooding irrigation about 1 cm above soil surface led to senescence and decrease in chlorophyll content of leaves. (Schelmer et al., 2005) [55] stated that drought stress had no significant effect on chlorophyll content of maize leaf and concluded that decrease in turgor pressure caused by water deficit, result in change in amount of far red radiation passed through the leaf and this reason, read of chlorophyll meter device was changed. In other words, light reflection from leaf was increased with increasing water stress (Barry et al., 1995) stated the same result in wheat. Also, (Fotovat et al., 2007) [20] found that by exerting severe drought stress on wheat, chlorophyll content of leaf significantly decreased. Chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield and facilitate selection of high yielding genotypes (Pandey and Singh, 2010) [47].

Proline Accumulation
Proline Accumulation of plants could be only useful as a possible droughts injury sensor instead of its role in stress tolerance mechanisms. However, (Vendruscolo et al., 2007) [66] found that proline is involved in clearance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of drought stress (Tatar and Gevrek, 2008) [63] showed that wheat dry mater production, RWC decreased and proline content increased under drought stress. Higher proline content in wheat plants after drought stress has been reported by (Patel and Vora, 1985; Pandey et al., 2001) [46]. It is also a rapid response to changes in aqueous condition. The exact mechanism of proline accumulation is related to the role of proline accumulation in plant underwater stress (Aspinal and Paleg, 1994) [9]. Revealed that the proline content increase as the drought stress progressed and reached a peak or recorded after 10 days stress and then decreased under sever water stress as observed after 15days of stress (Saeed 2013) [52]. To increase plant tolerance to abiotic stresses and maintain a high relative water content, plants may accumulate compounds of low molecular mass such as proline and gibberellins (Zlatev and Lidon, 2012) [71], possibly through buffering the cellular redox potential (Wahid and Close, 2007) [67]. The accumulation capacity of the compounds protects protein structures as cells dehydrates and is linked to genetic variability of plants for moisture stress tolerance (Monica et al., 2007) [43]. Some authors indicated that the relationship between proline accumulation and turgor could be a useful water injury sensor (Iannucci et al., 2000) [23], since during stress the proline level can be as much as 100-fold higher than in normal conditions (Bellinger and Larher, 1987) [10]. Most proline accumulates in leaves, and the least in the roots. Recent studies show that proline may differently affect the stress tolerance, increase the activity of many enzymes, and stabilize protein integrity (Figure. 2). Proline contributes to maintenance of the redox balance, can regulate development, and is a component of metabolic signalling networks controlling mitochondrial functions, stress relief, and development. In addition, biosynthesis of cuticular waxes in the aerial parts of land plants is also closely associated with water stress tolerant responses (Lee and Suh, 2013) [34].

Fig 2: Proline functions used for protein synthesis in plant has protective functions as an osmolyte, contributes to the maintenance of the redox balance, can regulate development and is a component of metabolic signaling networks controlling mitochondrial functions, abiotic stress relief and development. Abbreviations: PCD, Programmed Cell Death. CAT, Catalase; APX, Ascorbate Peroxidase (Szabo and Savoure, 2010).

Molecular responses
Analysis of the functions to stress inducible genes is an important tool not only to understand the molecular mechanisms, and the responses of higher plants, but also to improve with the stress tolerance of crops by gene manipulation. The expression of a particular gene in the wheat genotypes observed differences in its expression kinetics, thus confirming the high complexity of the mechanisms that regulate the expression of genes. A variation in gene expression exists among genotypes within both classes. A relation between DHN gene expression and time of stress was not possible because of the different behaviour of the genotypes analyzed in relation to water loss and analyzed the relationship between the DHN expression profiles and the
physiological data with particular attention to RWCs. The expression of the DHN genes parallels the RWC values, but differences were observed between drought resistant and susceptible genotypes. In resistant genotypes the induction of DHN genes is triggered when RWC values are still high (~74 to 91%), while in the sensitive genotypes the same genes is activated only when the water content drops to 59–65%. Although we cannot refer to a common RWC fixed value to measure gene induction, show assess that resistant and sensitive classes differ in their response of gene induction to stress. Analysis of WLRs also indicates that, in general, in resistant genotypes, although the water loss proceeds at a slower rate, the DHN gene expression is activated at WLRs lower than in the sensitive ones. The DHN transcripts accumulated during the stress also in the sensitive genotypes, but when WLRs were higher than those of resistant genotypes. In other words, in resistant genotypes the accumulation of the DHN transcripts, induced by drought stress when RWCs are still high, could be involved in the reduction of WLR. The water-retaining ability of DHNs was already suggested in other plant species (Wisniewski et al. 1999) [69]. Comparing DHN transcript accumulations and free proline content, then observed a relation between these data. In particular in resistant genotypes, all DHN transcripts start to accumulate at proline values lower than those of the sensitive genotypes. Because the free proline accumulation can be considered an index of plant stress perception so could hypothesize that in resistant genotypes there be a more efficient mechanism of stress perception. Nevertheless, considering that DHN genes are silent under normal conditions, we can suggest an extremely fine-tuning of DHN genes in comparison to cell and tissue responses, indicating the presence of a complex genetic control for drought resistance. This complexity was already suggested by Suprunova et al. (2004) [60] for barley, in which the authors hypothesized that higher drought stress resistance could be attributed to the combination of different factors such as high expression level of DHN genes, the presence of more efficient transcription factors, more efficient signal transduction and the cellular early perception of the stress. The complexity of the drought tolerance trait consequently implies that it cannot be genetically analysed as a monogenetic character. A way to dissect the analysis of this complex trait is the quantitative genetic approach such as detection and mapping of quantitative trait loci (QTLs) to identify the regions involved in its regulation (Teulat et al. 2003; Suprunova et al. 2004) [64, 60]. A molecular genetic approach could be useful to understand the genetic interaction between traits or collocation of QTLs with gene sequences. In fact, several QTLs controlling tolerance traits have been identified close to DHN genes (Campbell and Close 1997) [13] that, according to these authors, may be key genetic determinants of drought tolerance and for which possible roles in cell volume/turgor maintenance has been suggested (Teulat et al., 2003) [64]. Further investigations, including other DHN gene expression analysis as well as quantification of their expression, are needed to add new insight to the molecular aspects of drought tolerance in plants and to clarify the role of single members of DHN gene family in the general pattern of drought stress response. In addition, we have to consider that experimental conditions used in this work to induce drought stress already reported for other species, (Malatras et al. 2002; Liu and Baird 2003) [39, 36] account for the stress response in laboratory conditions, and in the future, the selected genotypes should be tested in the field to confirm their responsiveness to drought stress. Nevertheless, the results here reported are helpful to elucidate physiological and molecular aspects of the response of plants to drought stress.

**Conclusion**

Crop production under field conditions can be decreased by several abiotic stresses. This gives studies on multifactor interactions greater importance than analyses of only one stress. Plant reaction to a combination of the drought stress cannot be directly extrapolated from the response of plants to each of these different stresses applied individually. Co-occurrence of drought stress affects plants to a larger degree than the summary effect of stresses. Plant roots and shoots manifest numerous adaptive changes in response to drought stresses. The decrease in root hydraulic conductivity induced by drought reduces water flux into the plant, but also prevents water losses from the plant to the dry soil. Root growth under initial drought conditions is generally enhanced for a better access to water and dehydration avoidance. However, drought stress results in root shrinkage, anatomical deformations, and weak root soil contact that limits water and ion supply. In legume crops, the stresses decrease nodule size, weight and nitrogenase activity. The alterations in root growth and distribution reduces root growth and functions as an effect of root-to-shoot signaling with the contribution of plant hormones and other factors (nitrogen, calcium, pH) acting as signal molecules. The plant stress hormone, ABA, has long been recognized to act as a major chemical root-to-shoot signal under stresses. Drought stresses induce stomatal closure, decrease the transpiration rate and photosynthesis activity particularly through the effect on photosystem PS II, and lead to earlier crop maturity and poor productivity. Other responses include accumulation of compounds of low molecular mass (proline), anatomical deformations at the tissue, cellular, and sub-cellular levels. Expression of other proteins is an adaptation strategy to high temperatures. C3 (wheat) compared to C4 (maize) plants are more sensitive to drought stress. Soil-plant water relations and heat balance can be influenced by tillage, surface residue management or mulching through the effect on. The soil surface roughness, surface energy partitioning, gradients in temperature and water vapour, infiltration and amounts of water stored in the soil, and water uptake by the plants. The root drying approach has been recently applied to increase crop WUE and root WUE in many regions. Crops and varieties with good stand establishment and an extensive root system perform better in drought area. Foliar application of growth regulators can alleviate the adverse effects of drought stress through keeping an appropriate level of water in the leaves due to osmotic adjustment and stomatal performance. There is relatively little information about the effects of the drought stress along with other environmental threats, such as soil compaction, erosion salinity, and acidification. Further research involving determination of plant assimilate partitioning, and phenotypic plasticity is essential forunder standing the complexity of the responses and for breeding using molecular protocols or genetic engineering of plants that can tolerate abiotic stresses.

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