Indian J. Applied & Pure Bio. Vol. 38(2), 554-566 (2023). **ISSN: 0970-2091**
A web of Science Journal

Assessment of Physiological and Biochemical parameters of Barley *Hordeum vulgare* L. genotypes under Drought stress

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Abstract

Due to the current state of global warming and climate change, there are large geographical regions where plants are typically stressed by drought. It is the primary environmental influence that alter a range of morpho-physiological and biochemical characteristics, restricts plant development and agricultural yield. One of the most popular cereals and one with excellent water deficiency adaptation mechanisms is barley. To mitigate the difference between the actual and prospective yield in drought-prone locations, high yield cultivars with physiological and biochemical features imparting drought resistance must be adopted such as chlorophyll content, stem reserve mobilisation, canopy temperature, anti-oxidants and osmolytes. Through breeding and selection, these morphological, physiological and biochemical traits traits can be improved, resulting in genotypes that are more resistant to drought environment.

Key words : Drought stress, Barley, *Hordeum vulgare* Morphological, Physiological and Antioxidant indices.

With the rise of global population to over 9.7 billion people by 2050 and the unpredictable climate change pattern, it has become a challenging task to increase the crop yield[27]. Plant growth and productivity is severely affected by various biotic and abiotic stress factors; various anthropogenic activities have triggered these factors, thereby, limiting the crop productivity worldwide[54]. Abiotic stress is accountable for more the 50% decrease in the crop yield, causing loss of worth hundreds of millions of dollars per year[10]. Among these stresses, drought stress is the main limiting factor of plant growth and crop productivity[17,60]. According to Pachauri *et al.*,[68], as the surface temperature increases, heat waves with high intensity and longer duration will occur more often over most land areas on daily and seasonal time scales. As the intensity of the drought stress is escalating day by day, the world agricultural output is predicted to suffer catastrophic losses of up to 30% by the year 2025 compared to the present yield[4].

Water deficit have an impact on each yield component to varying degrees which depend on the stage of plant growth at the time these conditions exist[88]. In case of cereals, the occurrence of drought during the vegetative development cycle truly threatens the plant survival[90]. Drought stress during the grain filling period reduces the individual grain weight that leads to decline in the grain production[77]. In barley, the grain filling rate was dropped by 40% caused by water deficit during grain filling period[78]. At anthesis, moisture deficit greatly influences the number of grains per ear, thereby, decrease in yield[89]. According to Samarah *et al.*[77], drought stress has been shown to significantly lower grain yield by 49–87% in barley. Despite the decrease in the crop yield caused by water stress, barley is one of the most widely grown cereals that adapts best to the water deficit condition[53]. Barley is regarded as a significant cereal crop in many developing nations such as India, Nepal, Sri Lanka, and Bangladesh[16]. Barley is regarded as a model plant for morphological, physiological, and genetic studies because it is a true diploid plant with a high rate of self-fertilization (99%) and is easy to cross-fertilize. It also has a short growing season, taking only 2-3 months to complete its life cycle. It contains a vast genome of approximately 5.1Gb spread over seven separate chromosomes (2n=14)[17].

Drought stress is complex and its effects vary depending on the environment; traits or genes of the crop that aid in increasing the yield in extreme drought condition may not operate in moderate drought condition and may even have the opposite impact in well-watered situations[65]. Drought has an impact on nearly all phases of plant growth and development, resulting in decrease in photosynthesis, reduced transpiration, floral anomalies, spikelet/kernel sterility, decreased grain yields, and quality losses[44]. Water deficit can affect the

Table-1. Recent reports published on the effects of drought stress on different developmental stages of barley.

Developmental stage	Main research findings	References
Vegetative	Nanosilicon-based recovery of barley (*Hordeum vulgare*) plants subjected to drought stress.	Ghorbanpour *et al.*,[26]
Pre-anthesis	Effect of water deficit stress on physiological traits of some Algerian barley genotypes.	Hebbache *et al.*,[35]
Post-anthesis	Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolic QTLs for metabolites associated with antioxidant defense.	Templer *et al.*,[83]
Grain filling	The influence of drought stress on malt quality traits of the wild and cultivated barleys.	Hong & Zhang[38]
Seedling	Screening of worldwide barley collection for drought tolerance: the assessment of various physiological measures as the selection criteria.	Cai *et al.*,[11]

three key stages of barley crop development: 1. Vegetative (stage prior to the reproductive stage); 2. Pre-anthesis (period from tillering to flowering); 3. Post-anthesis (after flowering till maturity). Water deficit causes oxidative stress resulting in excessive electron leakage towards O_2 during photosynthetic and respiratory activities, thereby, increasing the production of reactive oxygen species and eventually cell death[19].

Reynolds *et al.*[71] stated that more and more regions of the world may likely experience semi-arid and arid climates in the coming years as a result of climate change, hence, information and understanding of traits associated to drought tolerance have become crucial for the investigation of crop tolerance mechanism[50]. Gous *et al.*,[28] demonstrated that genotypes that have better photosynthetic capacity and stomatal conductance are more resilient to drought stress.

Crop efficiency under drought circumstances including drought avoidance and drought tolerance is a highly complicated process because of some unforeseen environmental variables and their interaction with other abiotic and biotic components and various molecular, biochemical, agronomic and physiological phenomenon affecting plant growth and development[65]. Cai *et al.*,[11] suggested breeding of drought tolerant barley varieties to be the most efficient and cost effective strategy to reduce the negative impacts of drought stress on crop yield. In order to develop varieties that are resistant to a wide range of stress conditions, multiple stress-tolerance responses at different developmental stages of the plant are integrated[70].

Effect of drought stress on Barley :

Drought stress, according to several reports, is one of the most destructive to barley grain production, particularly during the post-reproductive stages, depending on the duration, intensity of the stress, and crop development phase[46,55] by interrupting the supply of carbohydrates from the source organs, which results in a large number of abandoned flowers and grains, a decrease in the weight of the individual grains, and a smaller seed size [46,81]. Drought stress restricts plant development by disrupting several morphological, physiological and biochemical functions such as photosynthesis, chlorophyll production, ion absorption and transport and carbohydrate metabolism[6,38].

Effect of drought stress on barley phenology:

Crop phenology is the most prominent feature of crop adaptation, and variations in the geographical and timing of phenological stages signifies the strong biological impact of drought stress[18]. The crop productivity and quality can be directly altered by phenological stages at which water deficit condition occurs[43]. According to Menzel *et al.*[61], any alterations in phenological timing have a great impact on the yield of cereal crop either directly or indirectly. Plants when faced by drought environment produced relatively fewer grains as a result of shorter period of phenological events such as booting, heading, anthesis and physiological maturity to complete their life cycle[82,83]. Even yet, droughts can affect both the vegetative and reproductive periods. The effects of high temperature on pollen viability, fertilisation, and post-fertilization stages result in a significant reduction in final yield[34]. However, not all phenological phases respond identically to drought effects nor have equivalent effects on yield and quality[13]. Ahmed[3] stated that the reproductive phase of a cereal crop, which includes the spikelet initiation, heading, and grain filling phases, is the phase of development that is most susceptible to environmental stress. Heading date is crucial for adjusting barley genotypes to various enviromental conditions; drought at late sowing may disrupt barley developmental processes, which typically occur from the flag leaf until maturity, leading in a decrease in plant height, number of tillers, fertile tillers, number of spikelets per spike, dry matter accumulation and grain output[40].

The relevance of early flowering/ heading as a drought escape strategy has been well shown, as have favourable associations between earliness and grain output under stress in cereals in the studies of Carter[12]. Fatima *et al.*[18] concluded that with a change in sowing date and the creation of cultivars with longer length of phenological events, the effects of drought stress can be somewhat mitigated. To maximise grain output, crop life-cycle timing must be coordinated to minimise growth stressors associated with drought stress during the sensitive phase for yield determination, which occurs before and during flowering[51]. The production of drought-tolerant cultivars is a foolproof method of minimising the negative impacts of the stress[21].

Effect of drought stress on barley physiology:

To forecast the plant response to stress, several morpho-physiological parameters interact and vary in their respective responses based on the extent and period of the water deficit[35]. Drought is attributed to a reduction in water content, stomatal closure, lower chlorophyll (chl) content, and decreased photosynthesis, as well as a decrease in cell enlargement, growth inhibition, and leaf senescence, which is an age-dependent degradation process of making sure the translocation of nutrients from older leaves to developing tissues and seeds and varies at developmental stages of barley[23,84].

Relative Water Content :

RWC is a crucial trait that assesses the water status of plants and represents the current metabolic processes occurring in tissues and correspond with drought tolerance; in comparison to any index of plants, RWC is a superior determinant of drought stress[14]. In response to drought stress, a reduction in the RWC has been observed in a wide range of plants and the relationship between RWC and barley grain output was unfavourable[75]. However, decrease in leaf water potential was relatively lower in drought-tolerant genotypes as they constitute large percentage of organic osmolytes which promote osmotic adjustments under moisture deficit environment and employed for drought tolerance[33,91].

Stem Reserve mobilization :

When water deficit condition persists, plants restrict their photosynthetic activity, and stem reserves aids to supply the nutrients to the sink, which is the growing seed, providing some stability to the crop yield[66]. The frequency at which reserves are remobilized to grain is a critical component in a water deficit situation since it is inadequate to compensate for the reduced grain filling period as a consequence of stress[31]. High fructan storage reserves, advanced mobilisation efficiency, strong sink strength, and prolonged grain maturity period assist tolerant cultivars in enduring the drought conditions[30].

Canopy temperature and canopy temperature depression :

Canopy temperature may be used to assess plant water status, which is a significant variable of plant growth and crop development in a water-stressed environment[49]. Evapo-transpiration, soil moisture, wind, plant metabolic activity, ambient temperature, air humidity, and constant radiation all influence canopy temperature[67]. In many aspects, canopy temperature is the optimal physiological selection characteristic as it can be measured instantly, conveniently, precisely, and economically to estimate the temperatures of various regions of plant[56]. Stomatal conductance, transpiration rate, crop water usage, leaf area index, root characteristics, and grain production are found to be associated favourably with CTD[23].

Chlorophyll-related traits :

The primary process in agricultural plants that produces dry matter and grain yield is photosynthesis and many researchers stated that monitoring photosynthetic characteristics like chlorophyll concentration might aid in understanding the impact of environmental stress on crop development and final productivity[58]. Chlorophylls are the essential pigments in the chloroplast antenna complex that absorb light energy to be converted into carbohydrates. Drought causes the breakdown of the thylakoid membrane within chloroplasts in most plant species, adversely impacting chlorophyll and other photosynthetic pigments[64]. Moisture deficit typically results in reduction of overall chlorophyll content and ratio of chlorophyll a/b to alter; Monteoliva *et al.*[63] reported that drought stress induces accelerated degradation of chlorophyll a which led to a decline in the chlorophyll a/b ratio in barley. Due to water stress, the reduction of chlorophyll a (Chl a), chlorophyll b (Chl b), membrane stability index (MSI), chlorophyll content index (CCI), water content in plant tissues, and nutrient availability (N. P, K) hinders the physiological performance of plants[25,59]. Under drought circumstances, a substantial positive association has been discovered by Istnbuli *et al.*,[42] between the Chl content, various Chl fluorescence characteristics, and yield in barley. The amount of chlorophyll in cereals was discovered to drop by 13–15% with increasing water deficiency[93].

Not all plants experience a decrease in chlorophyll concentration during water deficit, though. The potential to regulate chlorophyll concentration varies depending on genotype, as well as the period and degree of stress[63]. This stability acquired by the chlorophyll during drought stress is defined as chlorophyll stability index (CSI) and it is a function of temperature[69]. CSI is inversely proportional to the frequency of the drought stress. The tendency of barley to maintain high chlorophyll status during stress has been ascribed to a drought tolerance mechanism[29].

Effect of drought stress on biochemical parameters in barley :

Biochemistry and metabolism of plants are significantly altered by drought such as increased proline content, amino acid content, intensification of soluble sugars and enzymatic and non-enzymatic processes to mitigate the oxidative stress[14].

Cell wall polysaccharides and water soluble carbohydrates :

The plant cell wall's composite structure consists of a configuration of cellulose-hemicellulose encased in pectin-proteins matrix, facilitating it to adapt to a variety of stressful environments[62]. However, the implications of water deficit on cell wall mechanical performance, and alteration in the proportions of polysaccharides are projected to stunt the plant development[87]. Numerous researchers have revealed that hemicellulose and pectin concentrations rise as drought stress progresses[73]. After being photosynthesized in the leaves, sugars like glucose and fructose are transferred to the internodes where they are stored as water soluble carbohydrates (WSCs)[20]. Water-soluble carbohydrates (WSC) assimilates are accumulated in the internodes throughout the stem elongation till the commencement of grain filling, thereafter, being remobilised to the sink i.e. the growing seed during grain filling process constituting approximately 20% to the final grain yield under optimal conditions, whereas, more than 50% increase in grain productivity under drought stress[36,51]. Drought hinders photosynthesis, lowers the sucrose level, affects the translocation efficiency from source to sink, and also limits sink's potential to effectively use imported assimilates[17].

Anti-oxidants :

Most environmental stresses result in oxidative stress as a latter event in plants that leads to cell death attributed to substantial electron leakage towards O_2 during photosynthetic and respiratory functions resulting in the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydroxyl radical ($OH\cdot$), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2)[74]. ROS results in membrane damage, lipid peroxidation, protein denaturation, inactivation of enzymes, impaired photosynthetic processes, disruption of thylakoid membranes, damage to pigments, as well as a detrimental influence on plant development and finally programmed cell death[1,17,28,74]. Enzymatic and non-enzymatic antioxidant defence mechanisms have been devised by plants as scavenger for harmful ROS and limit oxidative injury; usually, enzymatic defence is regarded as the most effective[95].

Proline is vital in mitigating the negative consequences of drought by optimizing photosynthetic activities, strengthening cell membranes, hindering electrolyte leakage, enhancing accumulation of soluble sugar, is pivotal for osmotic adjustments and protects cellular components including chloroplasts, mitochondria, and DNA from oxidative damage during water deficit[1,35]. Proline also serves as an antioxidant, suppressing the proportion of reactive oxygen species to promote growth and development of plant[24]. Frimpong *et al.*[22] reported proline works as an osmoprotectant regulating the photosynthetic efficacy, growth, metabolic activity, and the final productivity under drought environment in barley. SOD, POD, and CAT enzymes either directly scavenge ROS or indirectly protect plants by influencing non-enzymatic defence[95]. SOD serves as the primary preventive measure by neutralizing superoxide radicals. Harmful O_2 radicals are transformed by SOD into H_2O_2, which is then scavenged by antioxidant enzymes like CAT and POD into O_2 and H_2O [37]. Enhanced SOD content under drought stress have reduced the potentially hazardous O_2^- levels[9]. The SOD, POD, and CAT activities of the drought-tolerant genotypes were relatively high than those of the drought-sensitive genotypes[75].

Malondialdehyde (MDA) level has been found to rise in response to generation of ROS, and this is a strong indication of oxidative damage imposed by drought stress[17]. MDA are frequently employed as a measure of lipid peroxidation driven by oxidative stress; lower MDA concentrations suggest less lipid peroxidation and high MBA represents high lipid peroxidation[79]. The level of MDA not only reveals the frequency and degree of lipid peroxidation but also the extent of tissue peroxidation injury. Plant resistance to abiotic stressors is correlated with the mechanism of ROS generation and its scavenging by strong antioxidative activity[39].

Total phenolic content :

Most often, plants have other counter-measure that lessens oxidative stress induced by drought *i.e.* biosynthesis of phenolic compounds[5]. Polyphenols, which include phenolic acids, flavonoids, and proanthocyanidins, constitute a significant and effective component in neutralizing free radicals or ROS[32]. Among the environmental stress, drought improve the volume of phenolic compounds in plants. Han *et al.*[32] reported rise in the total phenolic content during water deficit in barley crop. Moreover, a compatible association is demonstrated between biosynthesis of phenols and abiotic stresses in the latest findings[7].

Total protein content :

According to Kohl[48], protein accumulation depends on N reserves collected in the leaves and stems during the pre-anthesis period, which is mainly a restricted supply. Under drought stress at the anthesis stage, grain starch content dropped and protein content improved, but notably, drought-sensitive genotypes exhibited a markedly higher elevation in protein content[8]. Wu *et al.*[92] in his studies reported that when barley was treated to water stress following anthesis, yield components and protein content (PC) updated in opposite directions *i.e.* protein content increased and yield decreased. Six-row genotypes showed more decline in yield and protein content, indicating that they are more vulnerable to terminal drought than two-row barley genotypes[45].

Effect of drought stress on barley yield :

Drought can affect crops at any point in their life cycle, from the vegetative phase to the reproductive phases to the maturation phase[79]. Yield is essentially the complicated interconnection of several physiological mechanisms upon one another and drought stress has a deleterious impact on the majority of these physiological functions[89]. Water deficits might have an influence upon every yield component, the magnitude of which would depend on the stage of plant growth when these stress conditions emerge. A significant water scarcity during the seedling's early developmental phases might prevent them from emerging, growing, and developing fully, hence, hampering the grain productivity[85]. The preanthesis stage drought decreased the time and accelerated the pace to anthesis, while the postanthesis stage drought altered the duration of grain filling[17]. According to Cowley *et al.*[15], early development stage characteristics (for example, the number of tillers, biomass production, *etc.*) in a variety of cereal crops, including barley, are substantially closely linked with crop productivity potential and grain quality under optimal as well as stressed conditions. Drought stress decreased grain output in barley by cutting down the number of tillers, spikes, and grains per plant as well as the weight of each grain[86]. Due to fewer viable tillers, fewer grains, and a lower 1000 grain weight, a substantial decrease in the grain production of barley was noted during drought environments[17]. Moisture deficit lowers the agricultural crop output, particularly during the flowering and grain filling stages[1]. Grain weight, grain shape, and grain filling period all showed a substantial decrement with declining field capacity. Plants under immense stress mature ahead of time than plants provided with optimal conditions due to greater rate and shorter grain filling time causing poor transfer of photosynthates resulting in drop in final yield[41]. The genotypes that developed early flowering under water deficit condition generated better productivity because they had longer photosynthetic durations that contributed to grain filling than those genotypes that postponed their flowering phase. This early flowering not only reduced the duration of grain filling but also impacted the time needed for plants to grow vegetatively; lower plant height and biomass development as a result meant that the developing grains at the grain filling stage were not supplied with adequate photosynthates[4]. Furthermore, because of its adverse effects on floret production and fertility during the time of stem elongation, water deficit reduces the amount of grains per unit area[86]. Researchers may find it useful to discover the important features that influence crop production during drought stress by examining the correlations between yield and its subcomponents[94].

Abiotic stressors, drought stress in particular are a significant barrier limiting agricultural yield globally. Plants exhibit a wide range of adaptations (phenological, physiological and biochemical) to drought that are mostly represented by a variety of negative adjustments on its growth and development. Phenological stages such as booting, anthesis and physiological maturity shorten their duration, relative water content of the cells reduced, decreased chlorophyll content, inhibited photosynthesis, limited grain filling period and decline in grain yield is seen under water deficit conditions. Elevated MDA levels showed that the cell membrane had been disrupted, however high SOD levels scavenge ROS generated due to oxidative damage and minimised the cell injury. The reproductive development phase is the one that is most severely harmed; moderate stress during anthesis or the grain filling period might drastically decrease the crop output. Remarkable improvement has recently been attained in reducing the adverse implications of drought stress, either via the use of genetic strategies or the induction of stress tolerance. However, there is still space for development, for instance, little is known about the links between genes and the environment. New research should concentrate on creating genetically modified plants utilising molecular and biotechnological methods. Traditional and manual measurements must change in order to clearly, rapidly, and securely produce more scalable measurements with high-resolution in order to advance at the rate that is demanded by global growth.

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