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Physiological and Agro-Morphological Response to Drought Stress

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Abstract: Although, drought stress has been well documented as an effective parameter in decreasing crop production; developing and releasing new varieties which are adaptable to water deficit conditions can be a constructive program to overcome unsuitable environmental conditions. A good understanding of factors limiting yield now provides us with an opportunity to identify and then select for physiological traits, which increase drought tolerance and yield under rainfed conditions. Applying different physiological and agro-morphological tests to appreciate drought tolerance in plant leads to faster selection methods. Therefore, these characters can be used as an indirect selection criterion for screening drought tolerance plant materials, this strategy will lead to new cultivars with high yield potential and high yield stability that in turn will result in superior performance in dry environments.

Key words: Morphological traits · Chlorophyll fluorescence · Oxidative stress · Germination · Electrolyte leakage

INTRODUCTION

Drought is raising threat of world. Most of the countries of the world are facing the problem of drought. The insufficiency of water is the principle environmental stress and to enter heavy damage in many part of the world for agricultural products [1-5]. Among the environmental stresses, drought stress is one of the most adverse factors for plant growth and productivity [6, 7]. Drought stress can reduce grain yield, have estimated the average yield loss of 17 to 70% in grain yield due to drought stress [8]. Drought is a complex physical-chemical process, in which many biological macro molecules and small molecules are involved, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements [9-14]. The ability of a cultivar to produce high yield over a wide range of environmental condition is very important. Response of plants to water stress depends on several factors, such as developmental stage, intensity and duration of stress and cultivar genetics [15, 4]. The plant response is complex because it reflects over space and time the integration of stress effects and responses at all underlying levels of organization [16]. Improving drought resistance is, therefore, a major objective in plant breeding programs for rainfed agriculture in these regions [17-19]. Knowledge of genetic behavior and type of gene action controlling target traits is a basic principle for designing an appropriate breeding procedure for the purpose of genetic improvement. Hence, the success of any selection or hybridization breeding program for developing drought-tolerant varieties depends on precise estimates of genetic variation components for traits [20, 21]. It inhibits the photosynthesis of plants, causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus [22]. In addition, it inhibits the photochemical activities and decreases the activities of enzymes in the Calvin Cycle in photosynthesis [17]. Conventional plant breeding attempts have changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability. Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [16, 23].

Morphological and agronomic traits have a special role to determine the importance of each trait on increasing yield, as well as to use those traits at the breeding programs, which at least lead to improving yield and introducing commercial varieties under end seasonal drought stress condition [24]. Morphological characters

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include root length, spike number, grain number per spike, 1000 grain weight, awn length [25, 15, 4]. Wajid *et al.* [26] reported that wheat crop produces highest grain yield by applying irrigation at all definable growth stages. Because irrigation is an expensive input, farmer, agronomist, economist and engineer need to know the response of yield to irrigation. Furthermore, Jahfari [27] and Rafique [28] reported that yield and yield components are significantly increased within different wheat cultivars. Garavandi and Kahrizi [29] by evaluating 20 bread wheat genotypes reports that genotypes has higher genetic diversities for grain yield, spike number per square meter, number of seed per spike, spike density and awn length in comparison with other traits. Development of cultivars with high yield is the main goal in water limited environments, but success has been modest due to the varying nature of drought and the complexity of genetic control of plant responses [30]. In plants, a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions [31]. 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 [32]. Understanding plant responses to drought is of great importance and also a fundamental part for making the crops stress tolerant [33].

The purpose of this research was to study drought stress effect on some physiological and agro-morphological, so that responses of these traits to drought stress can be evaluated in resistance to drought stress.

Oxidative Stress and Antioxidant Defense Systems: When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as super-oxide ($O2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen are produced. These ROSs may initiate destructive oxidative processes such as lipid peroxidation, chlorophyll bleaching, protein oxidation and damage to nucleic acids [34, 17]. The antioxidant defenses appear to provide crucial protection against oxidative damages in cellular membranes and organelles in plants grown under un-favorable conditions [35]. Active oxygen species were considered to be important damaging factors in plants which exposed to stressful environmental conditions such as drought [36-38]. The antioxidant defense system in the plant cell includes both enzymatic (antioxidants), such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) [37, 39, 38] and non-enzymatic antioxidants including β-carotenes, ascorbic acid (AA) [40], α-tocopherol (α-toc) [41], reduced glutathione (GSH) [42]. Carotenes form a key part of the plant antioxidant defense system, but they are very susceptible to oxidative destruction. Β-carotene, present in the chloroplasts of all green plants is exclusively bound to the core complexes of PSI and PSII. Protection against damaging effects of ROS at this site is essential for chloroplast functioning. Here β-carotene, in addition to function as an accessory pigment, acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them [43, 23]. A major protective role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage [44]. To keep the levels of active oxygen species under control, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage [17]. Superoxide dismutases (SODs), a group of metalloenzymes, are considered as the first defense against ROS, being responsible for the dismutation of O^{2-} to H_2O_2 and $O^2_.$ CAT, APX, POD are enzymes that catalyze the conversion of H_2O_2 to water and O2 [17, 38]. Transformation of many plant genera for useful traits, such as oxidant-resistance is now routine [45]. Recently the involvement of H_2O_2 and SOD in regeneration of plants has also been proposed [46]. Hydrogen peroxide is commonly taken as an indicator of oxidative stress, because it is induced by activated oxygen species (AOS) and also influencing the level of lipid per oxidation [35]. However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and/or peroxidase (POD) to water and oxygen [37, 38].

Acclimation of plants to drought is considered to promote antioxidants defense systems to face the increased levels of activated oxygen species (AOS), which in turn, cause membrane damage by lipid peroxidation and indicated by malondialdehyde (MDA) content, which is one of the main parameters for evaluating membrane oxidation extent and are toxic for cells [47, 38]. The decline in CAT activity is regarded as a general response to many stresses [48, 49]. Ahmadizadeh *et al.* [38] with suited on 37 durum wheat landraces from Iran and Azerbaijan republic reported that the activity of SOD and CAT decreased in susceptible landraces, where as in resistant landraces SOD and CAT remained unchanged and in some cases they showed an increase under stress condition. In these genotypes (resistant) increasing SOD and CAT accompanied with ID decrease in the membrane. There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice [48], foxtail millet [50], tomato [51], sugar beet [52], oilseed rape [17], wheat [53, 54, 38] and barley [55].

Drought Stress and PSII Activity: Photosystem II (PSII) is highly sensitive to environmental limiting factors and PSII reaction center and its chemical reaction being adversely affected by drought stress [56, 57]. Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sink for PSII activity during mild drought [58]. It was shown that PSII functioning and its regulation were not quantitatively changed during desiccation. The CO2 molar fraction in the chloroplasts declines as stomata close in drying leaves. Havaux [59] has investigated the impact of various environmental stresses (drought, heat, strong light) applied separately or in combination on the PSII activity. The existence of a marked antagonism between physicochemical stresses (e.g. between water deficit and HT) was established, with a water deficit enhancing the resistance of PSII to constraints as heat, strong light. Similar results were obtained on bean plants [60]. Noctor *et al.* [61] provided quantitative estimation of the relative contributions of the chloroplast electron transport chain and the glycolate oxidase load placed on the photosynthetic leaf cell.

Chlorophyll Content: Drought stress produced changes in the ratio of chlorophyll 'a' and 'b' and carotenoids [44]. Chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield. Significant relationships between chlorophyll content and yield and yield components facilitate selection of high yielding genotypes [62]. Photosystem II (PSII) is highly sensitive to environmental inhibiting factors and water stress will damage its reaction centers severely. The chemical reaction of PSII is also affected strictly by water stress [63, 57]. Chlorophyll concentration has been known as an index for evaluation of source, therefore decrease of this can be consideration as a non stomata limiting factor in the drought stress conditions. There are reports about decrease of chlorophyll content in drought stress conditions [64]. Also, it is reported that chlorophyll content of resistant and sensitive cultivars to drought and thermal stress reduced. But resistant cultivar to drought and thermal stress conditions had high chlorophyll content [65]. Some study has demonstrated that chlorophyll content is positively correlated with photosynthetic rate [66]. Increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield [67, 68]. It has reported under drought stress rate of chlorophyll a to b has increased on wheat [69]. Decrease of chlorophyll content and water potential of soil has represented in plants such as sunflower [70] and Tobacco [71]. A reduction in chlorophyll content was reported in drought stressed cotton [72] and Catharanthus roseus [32]. The chlorophyll content decreased to a significant level at higher water deficits in sunflower plants [73] and in Vaccinium myrtillus [74]. Other reports have represented that drought stress did not have effect on chlorophyll concentration [75]. Pastori and Trippi [71] expressed that resistant genotypes of wheat and corn had higher chlorophyll content than sensitive genotypes under the oxidative stress. Ashraf *et al.* [69] also reported that drought stress will reduce concentration of chlorophyll b more than chlorophyll a.

Chlorophyll Fluorescence: The use of chlorophyll fluorescence from intact, attached leaves proved to be a reliable, nonintrusive method for monitoring photosynthetic events and for judging the physiological status of the plant [76]. Fluorescence induction patterns and derived indices have been used as empirical diagnostic tools in stress physiology [77]. Thus, PSII fluorescence can be regarded as a biosensing device for stress detection in plants. One of the most important parameters in rapid fluorescence kinetics is variable Fluorescence (Fv), i.e., the difference between maximal and minimal fluorescence (Fm-F0). The variable to maximum fluorescence ratio (Fv/Fm) is an indicative of potential or maximum quantum yield of PSII [78]. It is an important parameter of the physiological state of the photosynthetic apparatus. The declining slope of Fv/Fm is a good indicator to evaluate photo-inhibition of plants exposed to environmental stresses such as drought and heat, accompanied by high irradiance [57]. A promising approach is the use of chlorophyll fluorescence, a technique that can provide large amounts of data with a minimum of expertise and time and without injury to the plants. Chlorophyll fluorescence works on the principle that photosynthesis is one of the core functions in the physiology of plants. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the health and vitality of plants [79]. Chlorophyll fluorescence techniques are often used to detect environmental, chemical and biological stress in plant tissue [80]. According to Paknejad *et al.* [56] drought stress reduces the variable (Fv) and initial (F0) fluorescence parameters and quantum yield (Fv/Fm). Under dry conditions chlorophyll fluorescence was considered as a useful tool for screening and breeding of wheat cultivars [81]. Vazan [82] reported that drought stress reduces variable fluorescence (FV), initiative fluorescence (F0) and quantum yield (FV, FM).

Relative Water Content (RWC): Drought stress affects water status in plants. Relative water content is useful means for determining the physiological water status of plants [7]. Relative water content is the indicators of degree of drought stress. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. Obviously, stressed plants have lower RWC than non-stressed plants. RWC of non-stressed plants range from 85 to 90%, while in drought stressed plants; it may be as low as30% [83]. In studies that performed on 4 cultivars of bread wheat, RWC reduced to 43 percent (from 88% to 45%) by moisture stress [84]. Mationn *et al.* [85] represented a similar report as regards a drop in the amount of RWC in tolerant and sensitive cultivars of barley. Significant differences in leaf water potential and RWC were recorded among the tolerant and intolerant cultivars of wheat; results were consistent with Subrahmanyam *et al.* [86] and Tas and Tas [87]. Therefore osmotic regulation will help to cell development and plant growth in water stress. It is defined that decrease of relative water content close stomata and also after blocking of stomata will reduce photosynthesis rate. It is reported that high relative water content is a resistant mechanism to drought and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall [65]. Overall decrease in RWC under drought stress was highly significant in all the cultivars used, in accordance with Allahmoradi *et al.* [88] in Mungbean, Mohammadkhani and Heidari [89] in maize, Moaveni [14] and Farshadfar *et al.* [2] in wheat. While, Liu *et al.* [90] reported a gradual decrease in RWC after application of PEG treatment as water stress and Gonzalez *et al.* [91] has also recorded significant decrease in Leaf Ø and RWC in barley under drought stress. Shamsi [65] reported that with an increase in the Intensity of drought stress on wheat cultivars, there was a decrease in relative water content.

Cell Membrane Stability: Cell membranes are one of the first targets of many plant stresses [38] and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants [92]. These modifications occur mainly in drought sensitive plants and lead to a loss of semi permeable properties of the cell membrane, which is the main reason of metabolic damages developed in water stress plants. Therefore the integrity and stability of cell membrane in water deficit conditions can be considered a possible adaptive value indicative of stress resistance. Cell membrane stability may be determined through estimation of the extent of cell membrane damage in desiccated of leaf fragment in vitro whit a polyethylene glycol solution (PEG) and subsequent measurement of electrolyte leakage into aqueous medium [93]. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells [92, 38]. These tests determine the degree of cell membrane damage caused by stress based on electrolyte leakage from the cells. The technique is relatively simple, repeatable and rapid and requires inexpensive equipment, can be used on plant material from a variety of cultural systems and it is suitable for the analysis of large numbers of samples [77, 94]. However, despite its many advantages, electrolyte leakage was found to be markedly influenced by various experimental parameters, especially washing time of collected samples before PEG exposure [95, 96], intensity and duration of the PEG treatment [97] and duration of the rehydration period [98].

Saneoka *et al.* [99] and Azizi-e-Chakherchaman *et al.* [100] in Lentil studied the relationship between plasma membrane stability (obtained from EC measurement) and grain yield in stress and non stress conditions. They reported that plasma membrane stability in genotypes under stress was significantly lower than genotypes under non stress conditions. The cell membrane stability has been exclusively used as selection criterion for different abiotic stresses including drought and high temperature in wheat [101], rice [102] and sorghum [103]. The test to detect the integrity of cell membrane is called cell membrane stability (CMS) and was used to characterize drought resistance in plants [104, 92, 94, 57, 38].

Germination and Recovery Germination: Seedling emergence is one of the stages of growth that is sensitive to water deficit. Therefore, seeds germination, are prerequisites for the success of stand establishment of crop plants. Under semiarid regions, low moisture is limiting factor during germination. The rate and degree of seedling establishment are extremely important factors in determination of both yield and time of maturity [105, 106]. Crop establishment depends on an interaction between seedbed environment and seed quality [106]. It is critical to understand the seed germination ability of drought-tolerant forage species under drought stress and their recovery response when removed from drought condition. This information will help in the successful establishment of pastures in dry land. Stress tolerance of plants varies among species and their ecotypes [107]. Recovery germination of seeds in fresh-water after they were exposed to saline conditions has been investigated [108] to determine if seeds can remain viable after being exposed to hypersaline conditions [109]. One of the prerequisites to successful breeding for drought tolerance is availability of reliable methods for screening of desirable genotypes. Classical breeding may be complemented with laboratory method which is created models for simulation of water deficiency and drought conditions. In this respect, one of the most popular approach is to use high molecular weight osmotic substances, like polyethylene glycol (PEG), added to the medium for seed germination or plant/cell development [105, 106, 110-114]. Coefficient of velocity of germination (CVG) indices evaluates drought stress tolerance. Genotype with height coefficient of velocity of germination (CVG) is in stress condition. There are significant differences between laboratory data and drought stress tolerance, such as growth seedling, root length, root/shoot [115] and coefficient of velocity of germination (CVG) [106].

Germination in solution with high osmotic potential is one of the most important laboratory methods suggested for screening drought tolerance of crop plants. Good laboratory tests for screening genotypes have to show significant correlation with drought resistance [116]. Genotypic ability for high root to shoot length ratio contribute to drought tolerance. The efficiency of soil water uptake is by the root system therefore it is a key factor in determining the rate of transpiration and tolerance to drought. Water uptake by the root is a complex parameter that depends on root structure, root anatomy and the pattern by which different parts of the root contribute to overall water transport [117]. Germination in polyethylene glycol (PEG), measurements of root length or rooting depth and the survival or growth of seedlings which is subjected to osmotic, have been suggested for drought screening [106]. An increased root growth due to water stress was reported in sunflower [118] and Catharanthus roseus [32]. Takele [119] found to have differential responses of genotypes to variable soil moisture deficits for their specific seedling shoot and root lengths. Grzesiak *et al.* [120] noticed varietal differences in seedling growth and coleoptile length affected by drought simulated by a water solution of mannitol of chemical water potential of -0.3 and -0.6 MPa. The characters germination percentage and root to shoot length ratio showed considerable variability under stress conditions.

Effects of Drought Stress on Morphological Characteristics: It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth [23]. Morphological characters such as number of tillers, grain per spike number, fertile tillers number per plant, 1000 grain weight, peduncle length, awn length, plant height, spike length, kernel number per spike, grain weight per spike and etc. affect the wheat tolerance to the moisture shortage in the soil [18, 121, 4, 5, 122, 123]. Study of yield contributing components in respect of their genetic mechanism is very important for improvement in grain yield. Information regarding interrelationships between quantitatively inherited plant traits and their direct and indirect effects on grain yield is of great importance for success in selections to be conducted in breeding programs [124]. The yield components like grain number and grain size were decreased under pre-anthesis drought stress treatment in wheat [125]. In some other studies on maize, drought stress greatly reduced the grain yield, which was dependent on the level of defoliation due to water stress during early reproductive growth [126]. Water stress reduces seed yield in soybean usually as a result of fewer pods and seeds per unit area [127]. Heydari *et al.* [128] to study genetic diversity of different traits in 157 lines of double haploid bread wheat, indicated that their under-study lines have higher genetic diversity for last internode length, number of fertile spike per area unit, plant height, number of grain and grain yield per spike in comparison with other traits like grain volume weight, days to maturity, days to heading and days to anthesis. Bahari and Sabzi [129] Studying morphological traits with grain yield of durum and aestivum genotypes showed harvest index, no. grain in m2 and No. of grain spike traits most have a role in increasing yield.

Ahmadizadeh *et al.* [122] with suited on 37 durum wheat landraces from Iran and Azerbaijan Republic reported that under drought stress conditions there were positive significant correlations between the yield and the fertile tillers number per plant, spike length, awn length and number of grains per spike. Garavandi and Kahrizi [29] by evaluation of 20 bread wheat genotypes reported that genotypes have higher genetic diversities for grain yield, spike number per square meter, number of seed per spike, spike density and awn length in comparison with other traits. Saleh [130] also obtained the similar results. Asaduzzaman *et al.* [131] also believe that moisture stress reduces grain yield of mungbean and maximum negative effects of drought obtained with once irrigation during growth season. Rafiei Shirvan and Asgharipur [132] also obtained the similar results. According to Ashraf and Foolad [133] glycine betaine and proline by applying osmotic adjustment, reduce the negative effects of stress in the incidence of drought conditions.

CONCLUSIONS

Drought tolerance consists of ability of crop to growth and production under water deficit conditions. A long term drought stress effects on plant metabolic reactions associates with, plant growth stage, water storage capacity of soil and physiological aspects of plant. Achieving a genetic increase in yield under these environments has been recognized to be a difficult challenge for plant breeders while progress in yield grain has been much higher in favorable environments. Present results showed that plants in drought stress make changes in some of their physiological and biochemical features. Also, the results of this research represented, that drought stress causes low grain yield and in drought stress conditions that the cultivars that have more relative water content (RWC), chlorophyll content, cell membrane stability and activity antioxidant enzymes are more tolerance to drought stress.

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