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Water stress mediated changes in morphology and physiology of *Gossypium arboreum* (var FDH-786)

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Abstract: Abiotic stresses exert a substantial influence on growth and yield in plants; water stress is one of the most imperative abiotic stress factors. The study was carried out to elucidate the effect of drought stress on growth and physiology in *Gossypium arboreum.* Plants were grown in plastic bags and drought level (5% and 15% drought and control respectively) were maintained. The experiment was laid out in complete randomized design (CRD) with three replicates each control and drought stress. Forty five days old seedlings were imposed water stress for 10 days. Data of various morphological characters (plant height, root length, shoot length, fresh and dry biomass and root shoot ratio), physiological attributes (relative water contents and cell membrane thermostability) was recorded. The morphological and physiological attributes revealed significant differences among control and drought stress plants. Analysis of variance (ANOVA) for morphological characters revealed that plant height, root length, dry shoot weight, dry root weight, and root shoot ratio were found to be significant while fresh shoot weight and fresh root weight was found to be non significant. For physiological attributes both relative water contents and cell membrane thermostability were calculated as significant factors. The present study suggest that cotton variety FDH-786 execute well in drought tolerance as the plant biomass and root shoot ratio is the major selection parameters in the breeding for drought tolerance program. Nevertheless physiological attributes cell membrane thermostability and relative water contents are also the prognostic markers in the selection of crop plants against abiotic stresses.

Keywords: *Gossypium arboreum*, Drought, Morphological, Physiological

1. Introduction

Drought is a worldwide problem, and only 0.007% freshwater resources in the world surface could be utilized by human beings. However, the distribution of these limited freshwater is greatly uneven in the world. There are 100 countries or regions which is facing the problem of water deficit, among which 28 countries were listed the topest country under sever deficit [1]. Consequently, studies on the drought resistance of crops are a focus in the agriculture science at present. Of various abiotic stresses known in nature, drought stress poses a major threat to crop production because water is essential at every stage of plant growth from seed germination to plant maturation [2, 3], so any degree of water imbalance may produce deleterious effects on crop growth, but it depends upon the nature of crop species [4]. Keeping in view the considerable demand for food, crop improvement for drought stress tolerance is of prime importance. However, understanding about the morphological and physiological basis is vital to select and breed plants for improving crop water stress tolerance [5,6].

Cotton is one of the most important economy crops grown in rainfed and irrigated areas of the world. It is regarded highly by the governments not only in relation to people's lives, but also to the income of cotton farmers and the economic development of cotton planting zones, as well as to national textile supply and foreign exchange income. Many people consider cotton to be the purest fiber on earth, or the "fabric of our lives". Drought stress affects the cotton plants by limiting fiber yield and lint quality. Like other agricultural crops, then growth, development and performance of cotton is adversely affected by moisture stress. Cultivars are needed that can endure and recover from drought so as to minimize the losses in rainfed areas and to reduce the water needed in irrigated areas.

In Pakistan, cotton is an important agricultural commodity; being an exporting item it fetches a considerable amount of foreign exchange. In addition within the country cotton plant provides raw material to the expanding textile industry. Clearly the cotton crop is of immense importance in the economy of Pakistan. During summer season, the crop is extensively grown in the irrigated areas of southern parts of the Punjab province (so called "the cotton belt"), and Sindh province. Production of cotton in many areas of both Punjab and Sindh provinces is limited by inadequate amounts of water supply or small amount of rainfall during growth and development of cotton crop. Although there are many other reasons for low production levels in of cotton, decreasing ground water supplies and high energy costs are also emerging problems of cotton cultivation in the country. Thus, during recent years the cotton breeders throughout the world have started to develop cotton materials bringing genetic modification in the elite cultivars as parents of new populations, and also utilizing new germplasm in their breeding program. However, the research work is in the initial stages [5]. As argued previously, variability in drought tolerance can only be of value of it is effected by a significant genetic component.

Drought tolerance is a complex agronomic trait with multigenic components, which interact in a holistic manner in plant system [7]. The development of plant materials showing enhanced tolerance for water-stressed conditions, through breeding and selection, becomes easier and effective if variation exhibited for the character is genetically affected. Because of a general lack of genetic investigation on drought tolerance, information on the genetic basis of drought tolerance is not frequently available in the literature.

Availability of knowledge regarding identification of the specific traits that determine crop performance under water deficit conditions, and which one is amendable either through genetic transformation or conventional breeding approaches could help cotton breeders to create drought tolerant crop cultivars [8].

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 [9-11]. Stem length was significantly affected under water stress in potato [18], *Abelmoschus esculentus*, [12]. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions in pearl millet [11]. The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress [12]. The root dry weight was decreased under mild and severe water stress in Populus species [13]. An increase in root to shoot ratio under drought conditions was related to ABA content of roots and shoots [14-15].

Greater plant fresh and dry weights under water limited conditions are desirable characters. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production [16]. Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution [17]. Mild water stress affected the shoot dry weight, while shoot dry weight was greater than root dry weight loss under severe stress in sugar beet genotypes [18]. Reduced biomass was seen in water stressed *Petroselinum crispum* [19].

Water deficit has different effect on root growth [20] reported that drought-stressed cotton seedlings showed some increase in root length but reduced diameter. Inadequate soil moisture reduced cotton root elongation [21-22] while reduced root length density at 42 and 70 days after emergence [23]. Incorporation of increased seedling vigor rapid root system establishment and lower root-to-shoot ratios were recommended to improve drought tolerance in cotton [24].

The importance of root systems in acquiring water has long been recognized. The development of root system increases the water uptake and maintains requisite osmotic pressure through higher proline levels in *Phoenix dactylifera* [25-26]. The root dry weight was decreased under mild and severe water stress in *Populus* species.

Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as [27] that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential.

The cellular membrane thermostability (CMT) assay [28-29] is an indirect screening technique for heat tolerance and also provides a reliable measure of tissue tolerance to heat and drought stress [30-31]. The CMT assay has been successfully used to identify heat tolerant and susceptible genotypes in several crop species, including cotton [32]. Information on the genetic behavior of CMT and HTI in upland cotton has not been established but is imperative to understanding the genetic bases of the two traits and providing theoretical grounds for applied cotton breeding programs. Several studies suggested the effectiveness of cell membrane thermostability in terms of relative cell injury level in detecting genetic variability in heat tolerance in warm season crops [33]. This technique is simpler, quicker and less expensive than the whole plant screen.

The objective of the present study was to evaluate the effect of the drought on the available genetic variability associated with plant tissues and whole plant drought tolerance to decide cultivar potential as breeding objectives.

2. Materials and Methods

2.1. Plant Material, Growth Conditions and Water Stress Treatment

In the present investigation, morphological and physiological studies were performed to study the cotton variety behavior under water stress. Seeds of cotton genotype (*Gossypium arboreum* L.), namely FDH-786 of Desi-cotton was obtained from local germplasm center (CCRI, Multan). This work was carried out in the green house of the Center of Excellence in Molecular Biology,University of the Punjab, Lahore. Seeds were germinated in plastic bags (size 16.25 × 21.25 cm) containing 1kg soil, peat and sand (1:1:1) and grown under green house conditions. Temperature in green house was 30±2 °C at day and 25±2 ^{0}C at night with relative humidity approximately 45-50% and a photoperiod of 14h. Metal halide illumination lamps (400 W) were used to supplement natural radiation. Light radiation reached a maximum of 1,500µmpl m^2s^{-1} at the top of canopy at midday. The experiment was laid out in a completely randomized design with three replications of each experimental unit (Treatments viz; control and stress plants). Seeds were sown in 60 plastic bags (10 bags per replication). Four seeds were sown per bag. After 2 weeks of emergence, seedlings were thinned to one plant per bag. The plants were irrigated every alternate day with normal tap water. After 45 days from sowing, a cycle of drought was induced by stopping irrigating the plants for 15 days. The volume of pure water added to the pots was calculated periodically to maintain the plastic bags of stressed treatments at 5% gravimetric humidity (GH) and non-stressed treatments at 15% GH [34].

2.2. Morphological Parameters

The morphological parameters like plant height (cm), root length (cm), shoot fresh weight (mg plant^{-1}), root fresh weight (mg plant^{-1}), shoot dry weight (mg plant^{-1}), root dry weight (mg plant^{-1}) were recorded for control and drought stress plants. Randomly selected plants were uprooted carefully after 60 days of sowing to measure the seedling shoot length with meter rod in centimeters. The data for the root length were recorded from same seedlings uprooted for shoot length again with meter rod. Fresh and dry weights were measured with electronic weighing balance. The average of three plants from each replication was calculated. The selected individual plants in each replication of control and drought stress was separated into leaf, shoot and root. The plants were dried at 80^{o}C till constant weight was obtained. The root shoot ratio was computed on dry weight basis.

2.3. Physiological Parameter

2.3.1. Relative Water Content (RWC)

The relative water content (RWC) of leaves was measured with slight modification [35]. A fully developed and young leaf of control and drought stressed plant was taken and fresh weight of both treatments was recorded. All the samples were immersed in distilled water for 12h and turgid weight of each leaf was recorded. The leaves were then blotted dry and weighed prior to oven drying at 70^{o}C for 48h. The leaf relative content was calculated using the following formula:

Relative water content (%) RWC=[(FW –DW)/(TW–DW)]×100, where FW is the fresh weight, DW the dry weight, and TW is the turgid weight (weight after the leaf was kept immersed in distilled water for 12 h).

2.3.2. Cell Membrane Thermostability (CMT)/ Electrolyte Leakage

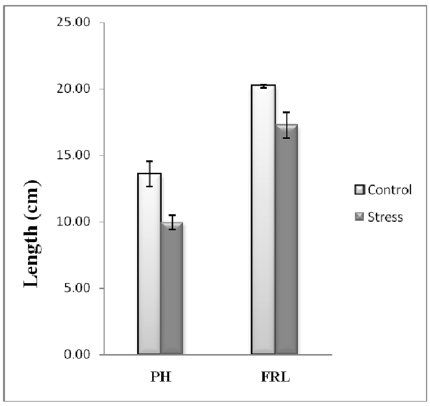
The protocol was employed to measure cell membrane thermostability of the leaf tissues [28]. Small discs of equal weight were cut from a fully developed 3rd leaf from each plant and placed in glass jars each having 20 cm^3 distilled H_2O, vortexed for 3 sec and initial electrical conductivity (EC_0) of each sample recorded. The samples were stored at 4 ^{o}C for one day and conductivity (EC_1) was measured again. Samples were then autoclaved for 20 min, cooled to room temp and EC_2 examined. The cell membrane thermostability was calculated as: Cell membrane thermostability (%) = (EC_1-EC_0) / (EC_2-EC_0) x 100

2.4. Statistical Analysis

Statistical analysis of the results was performed with STATISTIX V 9.0 (Analytical software Tallahassee, USA) freely online available. Graphs were plotted using Microsoft Excel. The data was subjected to one way analysis of variance (ANOVA) procedure for a complete randomized design (CRD).The least significant difference (LSD) test (P=0.05) was done to compare the means [36] and determine whether there were any significant differences for the parameters measured.

3. Results and Discussions

3.1. Morphological Characters



PH=Plant height, FRL= Root length

Figure 2. Comparison of plant height and root length in control and drought stress plants.

Under water stressed condition plant height (Figure 2) was markedly reduced. Data on plant height revealed that plants have differing response to the two conditions. Control plants had the tall plant height measuring 13.56 cm (Table 1). In contrast drought stressed plants had shorter plant height measuring 9.96cm. A reduction in soil moisture may reduce the availability of nutrients to the plant and consequently reduce plant height, growth and yield [37]. Drought stress reduces plant growth, so the carbon fixed during photosynthesis could be used to form secondary metabolites [38]. When plants experience drought stress, stem diameter shrinks in response to changes in internal water status [39].

Table 1. *Mean performance of control and drought stress plants for morphological and physiological characters.*

Morphological Characters	Treatments	
	Control	Drought
Plant height (cm)	13.567 a	9.966 b
Root length (cm)	20.200 a	17.260 b
Fresh shoot weight (mg)	1478.9 a	980.40 a
Fresh root weight (mg)	553.37 a	448.93 a
Dry shoot weight (mg)	323.63 b	453.47 a
Dry root weight (mg)	204.30 b	384.52a
Root shoot ratio	0.560 b	0.846a
Physiological attributes		
Cell membrane thermostability (%)	55.36 b	68.28 a
Relative water content (%)	70.23 a	44.30 b

Means followed by different alphabet are significant at 5% level of significance based on least significant difference test (LSD) while those followed by same letters are statistically non-significant

A B

Figure 1. *Cotton variety FDH-786 45 days old seedlings grown under controlled conditions.*

A row showing stress plants maintained at 15% gravimetric humidity
B row showing control (irrigated) plants maintained at 5% gravimetric humidity

Based upon root length (Figure 3) data (Table 1) plants didn't appear to response differently to control and drought stressed condition. The root length of control plant was 20.20cm. Under water stress significant reduction in root length (17.26cm) was observed. Extreme soil drying ultimately reduced root growth [40]. Root growth is less sensitive than leaf growth to the same tissue low water potential [41].The reason is in the greater osmotic adjustment in the extension region of roots as compared with leaves [42]. Drought stresses resulting from (Field capacity 15%) in cotton leads to reduction in stem and root growth, this reduction in root and stem length might be the result of roughening in cell wall.

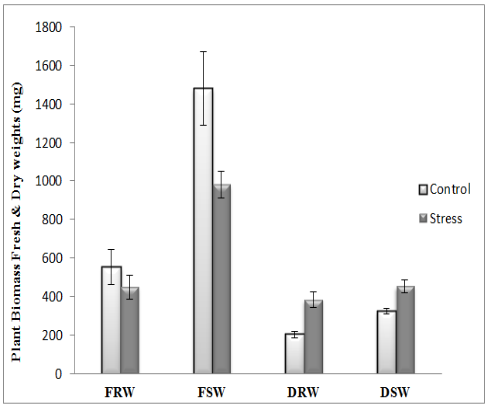


Figure 3. *Comparison of fresh and dry root and shoot weight in control and stress plants.*

FRW= Fresh root weight, FSW=Fresh shoot weight DRW= Dry root weight, DSW= Dry shoot weight.

Fresh shoot weight (Figure 3) and under control and drought stress condition didn't differ significantly. Data on absolute values of fresh root weight revealed that plants had no significant differential response under control and water stress condition. For control and drought stressed plant fresh shoot weight appeared to be 1478.9mg (Table 1) and 980.40mg respectively.

Dry shoot weight (Figure 3) of control and drought stressed plants differed significantly. The drought stressed plants had higher dry root shoot ratio as compared to the control plants. The differential response of plants to water stress for dry matter or weight is obvious from their indices of water stress tolerance. Shoot dry weight of 323.63mg (Table 1) and 453.47mg was observed for control and drought stress plants respectively.

Dry root weight (Figure 3) stressed plants was markedly increased in contrast to control plants. Control plants had less dry root weight measuring 204.30mg (Table 1) against dry root weight of 384.52mg in drought stressed which was found to be significantly higher as compared to control plans. Due to drastic increase in dry root weight in stressed condition the plants may be rated as tolerant to water stress.

Data on absolute values of root shoot ratio (Figure 4) revealed that stress plants had differential response to drought condition. Control plants had root shoot ratio of 0.560 (Table 1) while those of stress plants had a root shoot ratio of 0.846 which is significantly higher than that of control ones. Plants growing under stress condition with greater root shoot ratio may be called as drought tolerant plants in contrast to those plants growing under control condition. Under mild drought stress, pattern of resource allocation generally favors root growth rather than shoot growth. Severe stress conditions often decrease root growth. Timing of drought stress also has great influence on partitioning of carbohydrates and nitrogen. If drought stress occurs during early vegetative growth stages, there is a shift of partitioning toward roots rather than shoots, increasing the root-to-shoot ratio. This increase is due mainly to decreased shoot weight rather than increased root weight. Root mass rarely increases under stress, whereas root length and root volume often increase in response to mild stress. Generally, when water availability is limited, the root: shoot ratio of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials [43].

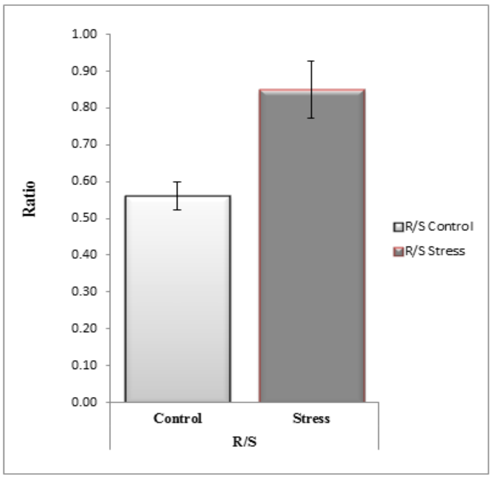


Figure 4. *Comparison of root shoot ratio in control and drought stress plants R/S= root shoot ratio.*

Analysis of variance for seedling traits under normal and water stress conditions is presented in Table 2. Variability was found in the material which was indicated by the presence of significant mean squares for the characters viz., fresh plant height, root length, dry root weight, dry shoot weight and root shoot ratio. While morphological traits fresh shoot weight and fresh root weight was found to be non significant.

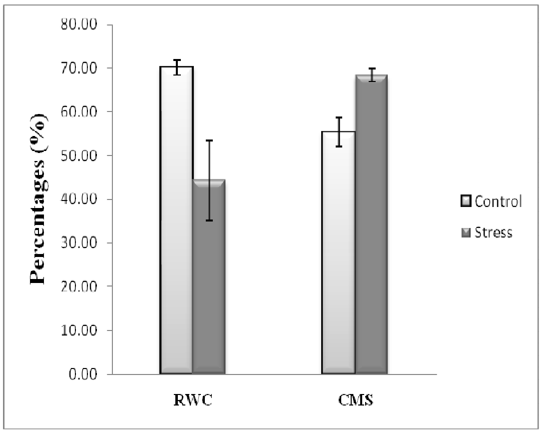
In present study the plant height was significantly reduced as compared to control plants. The reduction in height may be associated with a decline in cell enlargement under water stress. Water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions in pearl millet [44].

As far the root length is concerned it was found to be higher in control plants as compared to that of stressed plants. Root growth appears to be less affected by drought than shoot growth [45]. Our results illustrate [46] that growth and cell proliferation are separately regulated but often synchronized processes.

Greater plant fresh and dry weights under water limited conditions are desirable characters. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production [16]. Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution [17]. In current study, shoot and root dry shoot weight recorded were significantly higher in drought stressed plants than control plants. During the times of development, a fraction of assimilate partitioned to storage was higher in stressed plants than in comparison with the well watered plants.

When water supply is limiting, allocation of assimilates tend to be modified in favour of root growth and leads to increase root dry weight and consequently the root shoot ratio increases [47]. Although growth of both roots and shoots decreases under drought conditions the root shoot ratio generally increases. This is true because above-ground growth is affected more severely than below-ground growth. Root to shoot dry weight ratio in the present study increased as a result of water stress. The present study relates the findings of [47]. Thus, in result of drought stress on shoot and root growth and changes in dry matter partitioning, water shortage imposed at the whole stage of plant development then increased root to shoot ratio.

3.2. Physiological Attributes



RWC= relative water contents, CMS=Cell membrane thermostability

Figure 5. *Comparison of relative water contents and cell membrane thermostability in control and drought stress.*

Significant difference was observed for relative water contents (Figure 5) among between control and drought stressed plants. Relative water content was significantly higher in control plants as compared to stressed plants. In control plants water contents were found to be 70.23% while 44.30% (Table 1) was observed in contrast to drought stressed plants. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as [27] that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential.

Markedly increase in cell membrane thermostability (Figure 5) was found in case of drought stressed plants in contrast to those of control plants. In control and drought stressed plants the cell membrane stability was found to be 55.36% and 68.28 %respectively (Table 1). Adverse environmental factors cause cell membranes to lose selective permeability, cellular integrity and capacity for retention of intracellular substances [48]. The cellular membrane dysfunction due to water stress causes an increase in the permeability and ion leakage [49]. Thus increase in cell membranes leakiness is interpreted as an injury and loss of membrane integrity associated with a decreasing RWC, and this might accelerate senescence processes (Thompson, 1988).

Analysis of variance for physiological traits (Table 2) indicated that relative water contents and cell membrane thermostability mean squares were considered to be significant.

4. Conclusion

The conclusion is that although significant genetic variability existed for effective selection and genetic improvement of tissue and whole plant drought tolerance among control and drought tolerant plants for morphological characters (plant height, whole plant dry biomass and root shoot ratio) and physiological characters (relative water contents and cell membrane thermostability). Since relative water contents (RWC) and cellular membrane thermostability (CMT) are worthy pointers of whole plant drought tolerance, the advantage of using RWC and CMT as a rapid *in vitro* technique could be exploited efficiently in selecting whole plant drought tolerance. However, the RWC and CMT essay could be applied to reduce large segregating populations to a drought tolerant core for further evaluation and selection of agronomic and physiological traits in cotton plants.

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Table 2. *Analysis of variance (ANOVA) for drought tolerance indices based upon morphological and physiological characters.*

Morphological Characters	**Treatment MS**	**Error MS**	**F**	**P**	**CV (%)**
Plant height (cm)	19.44	1.73	11.18*	0.028	11.21
Root length (cm)	12.96	1.40	9.25*	0.038	6.32
Fresh shoot weight (mg)	372803	60797	6.13 NS	0.068	20.05
Fresh root weight (mg)	16359.5	17856.5	0.92 NS	0.392	26.66
Dry shoot weight (mg)	25285.0	1917.9	13.18*	0.022	11.27
Dry root weight (mg)	48720.7	3102.4	15.70**	0.016	18.92
Root shoot ratio	0.123	0.011	10.84*	0.030	15.16
Physiological attributes					
Cell membrane thermostability (%)	250.26	19.27	12.98*	0.022	7.10
Relative water content (%)	1009.07	127.24	7.93*	0.048	18.70

*, denotes significant differences at 5% probability level (P≤0.05) **, denotes significant differences at 1% probability level (P≤0.01) NS= non-significant
Treatment MS= Mean square (estimate of variance between groups), Error MS= Average of square of error value, F= Significance probability (variance ratio between Treatment MS and Error MS) , P=Probability value, CV (%)= Percent coefficient of variation

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