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Research Article

Physiological screening of cotton (*Gossypium hirsutum* L.) genotypes against drought tolerance

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Abstract

Cotton is a vital fiber and oilseed crop badly affected by soil moisture deficit stress. Screening of cotton germplasm is a prerequisite to classify the cotton genotypes as a drought sensitive and tolerant. With the aim to separate the distinct genotypes, eight cotton genotypes (BH-190, Ali Akbar-802, NIAB-86, NIBGE-115, FH-942, IUB-13, MNH-886, and FH Lalazar) were grown under normal moisture (08 irrigations/ 28 acre inches) and moisture stress (03 irrigations at 60, 90 and 120 DAS/ water deficit of 15 inches) environments. The assessment was done through different physiological parameters (stomatal conductance, osmotic and water potential, photosynthetic rate, relative water contents and canopy temperature). Cell injury and yield were also evaluated. The experimental design was Randomized Complete Block Design (RCBD) in factorial way with three replicates. The data obtained was investigated statistically at 5% probability and Least Significant Difference (LSD) test was used to isolate the significant means (treatment). The results indicated that water stress adversely reduced the values of all the above stated parameters. The cotton genotype BH-190 had significantly greater ($p<0.05$) yield and physiological attributes and found extra water deficit stress tolerant. While cotton genotype FH Lalazar had least value of these attributes as compared to all remaining cotton genotypes and thus considered as water deficit stress sensitive genotype. So, it can be concluded from the results of this experiment that the physiological screening of low soil moisture stress tolerant varieties could be a superior way to mitigate impact of drought stress on the cotton cultivated in drought susceptible regions.

Keywords: Cotton (*Gossypium hirsutum* L.); Drought tolerant; Physiological; Screening

Introduction

Cotton is an imperative cash crop raised for the fuel, fiber and feed [1]. Lint is a main product with various by-products i.e. cake, cotton-seed oil, linters and hulls. Cotton fiber has numerous uses from home material to fashion to medical produces. Cotton is eminent because of its usefulness, look and execution. After wheat, cotton is most noteworthy crop of Pakistan in terms of production, area, value addition etc. Pakistan has a special place in international cotton market due to its prominent contribution in that set up [2].

Cotton share to GDP and value addition of agriculture is 0.8 % and 4.5 % respectively.

During 2018-19, the production of cotton crop reached at 9.86 million bales with a decline of 17.5 % than the production of 2017-18 which was 11.94 million bales. During 2018-19, the production target of cotton was 14.40 million bales so the gap of 31.5 % existed between the achieved and targeted. Moreover, the amount of yield obtained during 2018-19 was 707 kilograms per hectare with a decline of 6.1 % than the yield of 2017-18 which was 753 kilograms per hectare. During 2018-19, the area under cotton cultivation was 2373 thousand hectare with a decline of 12.1 % compared to previous year's area which was 2700 thousand hectare (Govt. of Pakistan, 2018-19). Due to major contribution of cotton in GDP, cotton crop needs additional attention to safeguard them from hostile effects of water deficit stress by choosing drought tolerant varieties.

Due to adverse environmental conditions, a huge yield gap exists between the achieved and potential yield [2]. Among these adverse environmental conditions, drought is the key restrictive issue in crop production. Under drought stress condition, the rate of transpiration of plant is decreased and biomass accumulation in plant is linearly reduced [3]. So, resultantly, water stress often reduces plant growth and crop yield [4, 5]. Cell division, differentiation and expansion in cotton are the chief processes affected by drought stress trailed by decrease in the conductance of stomata [6]. Resultantly plant height, leaf area and photosynthetic rates are decreased while the rate of node production and squaring drop, resulting into yield reduction [7].

Under water deficit condition, nutrient availability and uptake decline and change the physicochemical processes of xylem sap [8]. Minor water deficit stress may raise the pH of xylem because of low uptake of nitrate causing raise in apoplastic pH [9]. Water deficit stress effects water content of leaf, rate of photosynthesis and water-use efficiency of plant [10]. Limited availability of water to crop before reproductive stage increases fruit withholding but abridged fruiting branches fruiting sites and nodes [11].

This experiment was conducted, keeping in view the present environmental condition, low and uncertain availability of canal irrigation water and significant reduction in yield per unit area, to screen the best drought tolerant variety of cotton among the most cultivated variety of cotton in Pakistan. Indirectly, this experiment had provided a best cotton variety with best genetic makeup which can be used further in breeding programs. So, in this experiment, the problems of both farmers and researchers community had been addressed.

Materials and methods

The study was conducted during kharif season in 2015 at Plant Physiology Research Area, Directorate of Agronomy, Ayub Agricultural Research Institute, Faisalabad (altitude 184 m, latitude 31.40º N, longitude 73.05º E). The trial was laid out in Randomized Complete Block Design (RCBD) with plot size of 3.0 m × 6.0 m. The R×R and P×P distance was retained 75 cm and 22.5 cm, respectively. The experiment was sown on May 19th, 2015. Fertilizer was applied@115-60-60 NPK kg ha^{-1}. Pendimethaline was used as pre-emergence herbicide for controlling weeds and all remaining field practices were retained constant. The eight cotton varieties (BH-190, Ali Akbar-802, NIAB-86, NIBGE-115, FH-942, IUB-13, MNH-886, and FH Lalazar) were grown under normal moisture (08 irrigations/ 28 acre inches) and moisture stress (03 irrigations at 60, 90 and 120 days after sowing (DAS)/ water deficit of 15 inches) environments. The assessment was done through different physiological parameters (stomatal conductance, osmotic and water potential, photosynthetic rate, relative water contents and canopy temperature). Cell injury and yield were also measured. The Procedure for data recorded were as follows:

1). Leaf Water Potential (-MPa): H_2O Potential is the movement of water molecules or free energy of water available to do work. Water always moves from high water potential to the lower H_2O potential. Leaf H_2O potential was assessed with the help of H_2O potential apparatus (Pressure chamber, Model 600, PMS International Company) by following the procedure **[3, 12]**. A single incised fresh leaf was stocked down in the chamber and the incised surface of leaf was kept out from the hole of the instrument. Gas pressure was given to the incised leaf through nitrogen gas cylinder until a bubble of xylem sap was appeared at the incised surface. This corresponding pressure was considered as tension prevailing in the sap of xylem and almost equal to the H_2O potential of plant cells. Plant sampling was completed up to 9.00 a.m. to evade from evaporation losses. Then leaves were immediately placed in the instrument to measure leaf water potential and all measurements were done on the flag leaf from treatments and control plots discretely.

2). Osmotic Potential (-MPa): The potential requires to molecules of water to pass from a dilute to a concentrated solution through a partially-permeable membrane. The leaf which was used for the measurement of water potential was frozen (-20^{o}C). The frozen leaf was thawed to remove the sap by pressing the leaf through glass rod or slab and extracted cell sap was poured into the Eppendorf tubes. A tiny drop of cell sap was employed unswervingly in presently calibrated osmometer (Cryoscopic osmometer, Osmomat 030-D, Genatec) for the measurement of osmotic potential. By using the following formula, pressure potential was calculated **[7, 10]**.

$\Psi p = \Psi \omega - \Psi s$

3). Stomatal Conductance (mmol $m^{-2}\ s^{-1}$): It is measured in m mol $m^{-2}\ s^{-1}$, which is the rate of carbon dioxide entering, or the rate of water vapor moving out via stomata. It was recorded on an intact leaf with the help of Porometer, an EGM-4 PP-Systems attached with a leaf chamber. Leaf having fully expanded blade was nominated for its measurements. Five measurements were recorded from five different plants in each treatment and then their average was taken. Transpiration and photosynthesis rate were also measured on leaf attached to the plant by using IRGA, infrared gas analyzer **[1-3]**. Five measurements were recorded from five different plants in each treatment and then their average was taken. 4). Canopy Temperature (°C): It is the direct recording of energy being evolved by the plant. It is measured by the IRIS, infrared temperature sensors. It provides information on plant water use, water status and exactly how plant is active metabolically **[1, 7]**.

5). Cell injury: In two sets, 0.1 g leaf samples were put in 10 ml of deionized water. One of the set was retained for 30 minutes at 40 ^{0}C and its conductivity was recorded (C_1) through conductivity meter. The remaining set was placed in a water bath for 15 minutes at 100 ^{0}C and its conductivity recorded as C_2. The cell injury was determined as:

Cell injury = (C_1/C_2) x100

6). Relative Water Contents (RWC) (%): RWC was recorded by using Schonfled *et al*. (1988). For the measurement of RWC, the top 2nd leaf of cotton shoot was detached through a sharp blade. The fresh weight of detached leaf was recorded immediately. To determine the turgid weight, these leaves were placed in the plastic bags containing distilled water. For imbibition, these leaves were left for 24 hours by retaining under the dim light whose intensity was about 20 m mol $m^{2}\ s^{-1}$ under natural conditions of laboratory. After that, leaves were removed and weighed to record turgid weight. Then leaves were placed in an oven at 70 Celsius for 72 hours to determine the oven dried leaf weight. All the recordings were done on a scale having precision of 0.0001 g. Following formula was used to determine the RWC.

RWC (%) = [(fresh weight - dry weight)/ (turgid weight - dry weight)]*100 7). Photosynthesis Rate: Net photosynthetic rate were estimated by gas analyzer (IRGA) in live plants. 8). Seed cotton yield (kg/ha): The yield was determined by number of pickings.

Statistical analysis

The recorded data was statistically analyzed via Fisher's analysis of variance (ANOVA) technique. LSD test was used ($p \leq 0.05$) to compare significant treatments means using Statistic version 8.1(Analytical Software ©, 1985-2005).

Results

The examined data for osmotic and water potential of eight varieties of cotton revealed that drought caused significant reduction in the water potential of all eight varieties of cotton (Table 1). However significant variation was perceived among cotton varieties under stress free treatments. FH Lalazar (0.7267) cultivar showed maximum water potential under well-watered conditions whereas BH-190 (1.1067) showed minimum water potential. Water potential of other verities i.e. Ali Akbar-802, NIAB-86, NIBGE-115, FH-942, IUB-13 and MNH-886 were intermediate under well-watered and drought environment. Under water stress, negative effects have perceived on osmotic balance and a plant tends to store different inorganic and organic molecules to decrease the osmotic potential. Under drought condition, osmotic potential significantly reduced. Under well-watered environment, highest value was shown by FH Lalazar (-0.233), MNH-886 (-0.243), IUB-13 (-0.263), FH-942 (-0.265) and NIAB-86 (-0.286), while cultivar with lowest osmotic potential included BH-190 (-0.298) and Ali Akbar-802 (-0.293) (Table 1).

Table 1. Effect of water deficit stress (DD) on water potential, osmotic potential, stomatal conductance and canopy temperature of eight cotton varieties

Cotton Varieties	Water potential (-MPa)		Osmotic Potential (-MPa)		Stomatal conductance (m mol m-2 s-1)		Canopy temperature (^{0}C)	
	WW	DD	WW	DD	WW	DD	WW	DD
BH-190	1.1067F	1.9433A	0.2987A	0.2770D	79.500A	58.983N	27.947G	30.117F
Ali Akbar-802	1.0767FG	1.9167A	0.2930B	0.2730E	77.667B	60.667M	28.353G	31.047EF
NIAB-86	0.9467GH	1.8967AB	0.2863C	0.2697G	75.567C	61.777L	28.687G	31.840DE
NIBGE-115	0.9167HI	1.8467ABC	0.2713F	0.2653H	73.947D	63.500K	28.967G	32.230CD
FH-942	0.8767HIJ	1.7667BCD	0.2663H	0.2597J	72.850E	64.417J	30.063F	32.597BCD
IUB-13	0.796IJK	1.7367CD	0.2633I	0.2533K	71.633F	66.137I	30.987EF	33.077BC
MNH-886	0.7767JK	1.7100D	0.2430N	0.2510L	69.417G	66.633I	31.727DE	33.583B
FH Lalazar	0.7267K	1.2833E	0.2333O	0.2470M	67.667H	67.707H	33.233BC	34.723A

The stomatal conductance of all the cotton varieties grown under water deficit condition was less than the cultivars grown in well-watered environment. However, BH-190 (79.500 m mol m-2 s-1) and Ali Akbar-802 (77.667 m mol m-2 s-1) have greater stomatal conductance than all other remaining varieties grown both under normal and water stress condition. The lowest stomatal conductance was observed in FH Lalazar (67.667 m mol m-2 s-1) and MNH-886 (69.417 m mol m-2 s-1) under normal condition.

Stomatal conductance shows the rate of transpiration and gas exchange through stomata. Greater conductance results in higher rate of transpiration and photosynthesis leads to higher crop yield. So, greater conductance of BH-190 and Ali Akbar-802 resulted in higher yield. Canopy temperature of plant decreases of those cotton varieties which contain higher conductance and transpiration rate. So the minimum canopy temperature among eight cotton varieties is BH-190(27.947^{0}C) and Ali Akbar-802(28.353^{0}C) under normal condition and BH-190 (30.117^{0}C) and Ali Akbar-802 (31.047^{0}C) under water stress (Table 2).

Table 2. Effect of water deficit stress (DD) on cell injury, relative water contents, photosynthesis rate and yield of eight cotton varieties

Cotton Varieties	Cell injury		Relative water contents (%)		Photosynthesis rate		Yield (kg/ha)	
	WW	DD	WW	DD	WW	DD	WW	DD
BH-190	26.963I	45.863A	70.347A	39.857I	16.680A	12.613H	2727.000A	1615.700G
Ali Akbar802	26.347J	44.627B	69.393B	38.643J	16.347B	12.287I	2636.700AB	1590.700GH
NIAB-86	25.487K	43.967C	68.720C	37.660K	16.153C	12.023J	2518.700BC	1565.700GHI
NIBGE-115	24.843L	43.340D	67.897D	36.87 L	15.980D	11.917J	2405.300CD	1535.700GHIJ
FH-942	24.320M	42.683E	66.670E	36.117M	15.797E	11.673K	2374.700DE	1489.00HIJK
IUB-13	23.600N	42.350F	66.213F	34.647N	15.560F	11.367L	2305.300DEF	1448.700IJK
MNH-886	23.130O	41.497G	65.397G	33.850O	15.440F	11.257LM	2269.000EF	1416.700JK
FH Lalazar	22.640P	40.913H	64.540H	33.407P	15.120G	11.170M	2198.300F	1388.300K

Cell injury is reliable parameter for the screening of plant varieties against abiotic stress resistance. Cell injury in well-watered plants was less as compared to water deficit varieties. The cell injury in BH-190 (26.963) in well-watered varieties and in drought stressed cultivar was BH-190 (45.863). Minimum cell injury was observed in FH Lalazar (22.640), MNH-886 (23.130).

In cotton, the loss of water through leaves is significant phenomenon under water stress. Rolling and wilting of leaves is result of water loss. Under drought, relative water contents (RWC) reduced significantly. Maximum relative water contents were observed in BH-190, Ali Akbar-802, NIAB-86 and NIBGE-115 with RWC values 70.347, 69.393, 68.720 and 67.897, respectively under normal condition which was higher than the varieties grown under water deficit condition. Maximum photosynthesis rate were observed in BH-190 (16.680), Ali Akbar-802 (16.347) and NIAB-86 (16.153). Due to high photosynthesis rate, yield of these varieties were higher as BH-190 (2727.0 kg/ ha), Ali Akbar-802 (2636.7 kg/ ha) and NIAB-86 (2518.7 kg/ ha).

Discussion

It is clear that assortment of suitable genotypes from large or small germplasm, biochemical and morphological attributes is a sustainable way that endorses the crop upgrading for soil moisture deficit stress tolerance **[13, 14]**. The different cotton progenies used in this experiment had showed different potentials to tolerate drought stress but on an average the most drought tolerant variety was BH-190 and the drought sensitive variety was FH-Lalazar as it showed the least tolerance to the observed parameters.

The results of another experiment showed the same as the current experiment had revealed that the water potential of cotton decreased when it was subjected to drought **[3]**. The results of various experiments demonstrated that the water potential and osmotic potential of different cotton varieties decreased when these varieties shifted from normal soil moisture condition to water deficit condition, resultantly, their turgor pressure decreased and the varieties with the satisfactory water and osmotic potential were considered as drought tolerant **[7, 15, 16]**.

Rate of photosynthesis is controlled by the stomatal conductance. The results of present experiment showed that the rate of photosynthesis decreased as stomatal conductance decreased. All the cotton genotypes had more stomatal conductance when cultivated in normal soil moisture condition than the water deficit condition which is consonance with the results of various experiment [15, 17-19]. Another study reported that the rate of photosynthesis and stomatal conductance was significantly affected in cotton under water deficit condition [20].

As indicated in the above results that canopy temperature of cotton genotypes was lower under well-watered condition than drought stress but in few genotypes the canopy temperature difference between two soil moisture condition was minor and similar type of results were also mentioned [21, 22]. From the above results, it was cleared that the relative water content of different cotton genotypes was nearly double under normal irrigation condition than the soil moisture deficit condition. Only those genotypes had showed high relative water content under drought stress condition which was able to tolerate drought and somehow similar results was also mentioned in other experiments [23, 24].

Cell injury is a reliable factor for the screening of drought tolerant plant. Under normal supply of irrigation water, plant cell membranes remain intact but under drought stress condition, the membranes of plant cells are damaged due to high reactive oxygen species, ROSs, concentration which leads to the release of cell content. That's why the above results showed the less value of cell injury in case of well-watered condition than drought condition and such type of results were also mentioned in other literature [2, 25]. The yield of seed cotton per plant actually determines the amount of seed cotton per hectare. The high seed cotton yield was recorded in those genotypes whose photosynthetic rate is also higher than the other genotype. There was a significant difference in the seed cotton yield of the same genotypes by only changing their soil moisture condition. From the above results it is concluded that seed cotton yield always reduces when cotton is grown in drought condition and the present result is in accordance with the other experiments results [26, 27].

Conclusion

It is concluded that under drought stress conditions (03 irrigations at 60, 90, 120 days/ water deficit of 15 inches), genotype BH-190 performed better with lower stomatal conductance (58.98 mmol m^{-2} s^{-1}), less canopy temperature (30.117 °C), low water potential (-1.9433 MPa), more osmotic potential (-0.2770 MPa), more cell injury (45.863), high relative water content (39.857), high photosynthesis rate (12.613) and high yield (1615.7 kg/ha) than all other genotypes under consideration. Whereas genotype FH-Lalazar performed poor and gave higher stomatal conductance (67.707 mmol m^{-2} s^{-1}), more canopy temperature (34.7 °C), more water potential (-1.2833 MPa), more osmotic potential (-0.2470 MPa), less cell injury (40.913), low relative water content (33.407), low photosynthesis rate (11.170) and low yield (1388.3 kg/ha).

Authors' contributions

Conceived and designed the experiment: A Ahmad & N Iqbal, Performed the experiment: A Ahmad, MZ Ilyas & Z Aslam, Analyzed the data: S Rehman & MZ Ilyas, Contributed reagents/ materials/ analysis tools: FA Marri & KN Khan, Wrote the paper: MZ Ilyas, A Ali, S Naeem & M Nazar.

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