Assessment of drought tolerance in various cotton genotypes under simulated osmotic settings

Muhammad Riaz Gondal1*, Muhammad Yasir Saleem2, Sultan Ahmad Rizvi1, Aaqib Riaz3, Waqas Naseem1, Ghulam Muhammad1, Sikandar Hayat4, Mazher Iqbal5
1Soil and Water Conservation Research Institute, Chakwal, Pakistan
2Fauji Fresh and Freeze, FFC Limited, Sahiwal, Pakistan
3Hussain Park, Military Farm Road, Sargodha, Pakistan
4Fodder Research Institute, Sargodha, Pakistan
5Barani Agricultural Research Institute, Chakwal, Pakistan

Abstract
Pakistan’s agriculture, especially the cotton area is facing serious threat of water shortage, which is negatively affecting the sizeable foreign reserves. Besides other irrigation management practices, selection of drought tolerant varieties can support to tackle the issue. The current study was aimed at the assessment of drought tolerance potential of various Bt cultivars of Gossypium hirsutum L. Under the current study, sixteen cotton cultivars were placed for germination in petri dishes under distinct osmotic potentials with seven different concentrations of PEG-6000 (i.e., 0, 5, 10, 15, 20, 25 and 27 percent, having osmotic potential of 0.0, -0.05, -0.148, -0.295, -0.491, -0.735 and -0.846 MPa respectively). The results revealed significant differences among various traits of all genotypes. It was observed that seed germination and root length increased up to concentration level of 25% PEG-6000 (at -0.735 MPa) whereas increment in shoot length stopped further. Root/shoot ratio increased until PEG concentration of 20% and then ceased. NIBGE-8 was the best performer under all simulated osmotic adjustments with maximum mean germination percentage of 62.86%. The growth parameters of NIBGE-8 recorded on 12th and 18th days after sowing were noted as root length (6.87 and 9.9) cm, shoot length (5.9 and 6.37) cm, root/shoot ratio (1.03 and 1.23), root length-index (597 and 843) and shoot-vigor index (539 and 576) respectively. The results of study revealed that the genotypes NIBGE-8, NIBGE-9, BH-201 and RH-668 were found osmotic stress tolerant while Mubarak, CEMB-88 and DEEBEL were found highly sensitive to drought conditions.

Keywords: Bt Cotton, PEG-6000, Osmotic potential, Drought resistance

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Introduction

Cotton (Gossypium hirsutum L.) is the essential fiber crop of the world, highly demanded all over the world. Nearly 70 nations of the world grow cotton for their domestic use and/or for export purposes. It is grown mostly in warm as well as mild zones of the world. During 2019-20, cotton was cultivated on about 2.5 million hectares, having annual production of 9.86 million bales (480 lb, bale) with average yield of 618 kg ha\(^{-1}\) (Economic Survey of Pakistan, 2020). Its contribution in GDP is 0.8% while its share in agriculture value addition is 4.1%. Harvesting of cotton is done by picking the cotton bolls from which seed is separated after ginning. Seed is utilized for extraction of oil and pressed seed is used for feeding livestock. Remaining fiber known as ‘lint’ is the entity of great importance which is further processed for the production of thread and ultimately for outfits production. Large yield gaps exist between production and yield potentials. These yield gaps are normally due to poor cultural practices, shortage of quality seed and lack of inputs (fertilizer, insecticide and irrigation water) as well as biotic and abiotic stress especially the drastic environmental influences such as extreme drought and high temperature regimes. It is said that in future cotton will have to be grown under extreme water shortage and high temperatures in relation with other abiotic stresses (Dabbert and Gore, 2014). Cotton growing areas in Pakistan are mostly warm and arid regions having little rainfall in addition to high temperature during season of cotton (Riaz et al., 2013). Cotton yield depends upon various factors such as potential of cultivar, environmental fluctuations and overall cultural practices. These factors influence the crop individually as well as in combination, which ultimately decreases yield significantly (Romagosa and Fox, 1993).

Abiotic stresses especially drought and heat mutually creates adverse survival circumstances in the life cycle of cotton crop plant. Cotton crop has the ability to withstand under drought conditions but for higher yields it need adequate water supply of about 2,158 to 3,906 m\(^2\) every growing season (McWilliams, 2003). Thus, yield and production of cotton increases with intense rainfall pattern. Few developmental stages such as flowering initiation stage and boll developing phase require sufficient water supply (McWilliams, 2003). Plant needs water at different developmental stages during its life cycle, which critically depends on plant water losses via transpiration and moisture quantity present in soil (Allen et al., 1998). Similarly plant developmental stage and time of irrigation as well as drought situation during cotton growing period, states the up and downs in the yield (Boman and Lemon, 2006). If drought stress remains for longer periods it effects plant height due to which short statured plant could be seen in field as compared to plant having sufficient water supply (Pace et al., 1999). Cotton boll weight, formation of seed inside the boll, seed and lint index as well as staple size, consistency, fiber development and its longevity are highly influenced under water stress circumstances (Wen et al., 2013).

Though cotton genotypes are significantly adjusted in particular environment and their selection is done after observing maximum potential in accordance to each specific desirable trait but, when it comes to shortage of water, these overwhelming challenges become hurdles in achieving maximum lint and seed yield which ultimately decreases the overall production. Survival of crop plant and increment in yield under such circumstances can be achieved by cultural and management practices such as by raising cover crops, sowing on beds, adopting zero tillage and using high efficiency irrigation practices to maintain soil moisture and tackle the water shortage at crucial stages. Besides these practices, selection of drought tolerant variety is vital to cover the gap of production. Keeping in view, these obstacles in cotton yield, the current study was planned to assess the drought tolerance potential of various Bt cotton cultivars using osmotic concentration technique.

Material and Methods

Experimental site and design

The experimental study was conducted to determine the effect of drought stress on yield and yield attributes of various cotton cultivars in the Laboratory located at the Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University D.I Khan during the year 2017-18. The experiment was laid out under completely randomized design (CRD) having factorial arrangement with three replications.

Treatments and data recording

Sixteen cotton cultivars viz., BH-201, CEMB-55, CEMB-88, CIM-602, CIM-625, CIM-632, CYTO-179, CYTO-313, DEEBEL, FH-142, FH-152, FH-153, FH-154, FH-155, FH-156, and FH-157 were used for the present study. The treatments were arranged in factorial arrangement with three replications.
Muhammad Riaz Gondal et al.

326, Mubarak, NIBGE-8, NIBGE-9 and RH-668 were selected for assessment of their tolerance against drought.

Ten healthy, identical and lint free seeds of each cultivar were chosen. These seeds were placed for germination, one at bottom and other on top of filter papers in sterilized petri dishes to maintain concentrations at 27°C. The seeds were placed apart in each petri dish under seven different concentrations levels of PEG-6000 i.e., 0, 5, 10, 15, 20, 25 and 27% that generated osmotic potential of 0.0, -0.05, -0.148, -0.295, -0.491, -0.735 and -0.846 MPa respectively. The concentrations were applied in such a way that 2 mL of each concentration at bottom and 1 mL on upper side of filter paper were applied. Then the petri dishes were transferred to incubator having temperature set at 27 °C. In order to maintain the levels of osmotic potential each PEG-6000 concentration was applied to petri dishes after an interval of 48-72 hours. The PEG-6000 concentration required to achieve a specific osmotic potential was computed by the formula given by Michel and Kaufmann (1973) expressed as:

$$\Psi_s = -(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C_2 + (2.67 \times 10^{-4}) \times C \times T + (8.39 \times 10^{-7}) \times C_2 \times T$$

Where:
\(\Psi_s\) = osmotic potential (bar);
\(C\) = concentration (g L\(^{-1}\) PEG-6000 in water);
\(T\) = temperature (°C).

For control, a solution with osmotic potential \(\Psi_s = 0.0\) MPa was used.

All the treatments were observed for germination (%), shoot length (cm) and root length (cm) recorded at 12\(^{th}\) and 18\(^{th}\) day after sowing (DAS) and implementation of PEG-6000 concentrations. Mean values of all three parameters were calculated and presented in data tables. Various plants growth parameters were investigated as described below:

**Germination (%) age**: Sprouted seed in each petri dish were considered as germinated seed and counted on 12\(^{th}\) and 18\(^{th}\) day after sowing. The germination percentage was computed as follows:

Germination (%) = \(\frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100\)

**Root and shoot length (cm)**: Five seedlings were taken out at random from each petri-dish without disturbing the root of seedling on 12\(^{th}\) and 18\(^{th}\) day after sowing and the longest root was measured in centimeters from the collar to the tip and recorded. Similarly, the shoot lengths were also measured for each treatment and recorded accordingly.

**Root to shoot ratio**: Root lengths and shoot lengths recorded on 12\(^{th}\) and 18\(^{th}\) day after sowing were converted into root-shoot ratio as follows:

Root-shoot Ratio = \(\frac{\text{Root length}}{\text{Shoot length}}\)

**Shoot and root vigor index**: Shoot and root vigor indices were also computed at 12\(^{th}\) and 18\(^{th}\) day after sowing using the expression proposed by Abdul- Baki and Anderson, (1973).

Root length index (RLI) = Root length \times Germination %

Shoot length index (SLI) = Shoot length \times Germination %

**Statistical analysis**

All the data gathered were subjected to statistical analysis following Fisher’s analysis of variance method (Steel, 1997). Means were compared with LSD test at 5 % probability level as described by Gomez and Gomez (1984).

**Results**

All the data collected under the study were analyzed statistically. The calculated variances showed highly significant differences among the genotypes as well as the interactive effects among all the parameters investigated. These results are presented in following tables (Table-1 & Table-2).

### Table-1. Analysis of variance for germination percentage

| Source          | DF | SS   | MS    | F     |
|-----------------|----|------|-------|-------|
| Genotype        | 15 | 8871.43 | 591.429 | 6.4E+30** |
| PEG             | 6  | 473239 | 78873.2 | 8.5E+32** |
| Genotype*PEG    | 90 | 6503.57 | 72.2619 | 7.8E+29** |
| Error           | 224| 2.09E-26 | 9.31E-29 |       |
| Total           | 335| 488614 |       |       |

** means highly significant
Table-2. Mean squares for various seedling related traits

| Source          | DF | RL   | SL  | RSR | RLI | SVI  |
|----------------|----|------|-----|-----|-----|------|
| Genotype       | 15 | 40.552** | 15.4278** | 0.12113** | 381373** | 162312** |
| PEG            | 6  | 2622.59** | 1942.94** | 78.9533** | 21190000** | 18510000** |
| Growth stage   | 1  | 1173.43** | 161.171** | 0.08371** | 6717600** | 848806** |
| Genotype*PEG   | 90 | 2.43476** | 1.35541** | 0.09697** | 36570.8** | 18408.1** |
| Genotype*Growth stage | 15 | 0.28686** | 0.82599** | 0.07628** | 5162.84** | 4589.14** |
| PEG* Growth stage | 6  | 82.382** | 24.533** | 7.28871** | 735968** | 175237** |
| Genotype*Growth- stage*PEG | 90 | 0.18196** | 0.32518** | 0.06594** | 1823.59** | 1867.36** |
| Error          | 448| 2.26E-30 | 9.95E-31 | 5.41E-32 | 1.27E-26 | 1.25E-26 |
| Total          | 671|       |      |     |     |      |

** means highly significant

Germination percentage

It was observed that the germination percentage reduces as the PEG-6000 concentration increases and became zero at concentration level of 27%. Mean germination values at PEG-6000 concentrations levels of 0%, 5%, 10%, 15%, 20%, 25% and 27% which generated osmotic potentials of 0.0MPa, -0.05MPa, -0.148MPa, -0.295MPa, 0.491MPa, -0.735 MPa and -0.846 MPa respectively were observed as 100%, 89.375%, 80.625%, 66.875%, 35%, 5.625% and 0% respectively (Table-3).

Table-3. Effect of different concentrations of PEG-6000 on germination % of various cultivars

| Genotype | Seed germination (%) at various PEG Concentrations |
|----------|--------------------------------------------------|
|          | 0%  | 5%  | 10% | 15% | 20% | 25% | 27% | Mean  |
| BH-201   | 100 | 90  | 80  | 70  | 40  | 10  | 0   | 55.71 |
| CEMB-55  | 100 | 80  | 80  | 70  | 40  | 10  | 0   | 52.86 |
| CEMB-88  | 100 | 90  | 80  | 70  | 40  | 10  | 0   | 55.71 |
| CIM-602  | 100 | 80  | 70  | 60  | 30  | 0   | 0   | 48.57 |
| CIM-625  | 100 | 80  | 80  | 60  | 20  | 0   | 0   | 48.57 |
| CIM-632  | 100 | 90  | 80  | 70  | 40  | 10  | 0   | 55.71 |
| CYTO-179 | 100 | 90  | 80  | 60  | 20  | 0   | 0   | 50.00 |
| CYTO-313 | 100 | 80  | 70  | 50  | 20  | 0   | 0   | 45.71 |
| DEEBEL   | 100 | 100 | 90  | 80  | 50  | 20  | 0   | 62.86 |
| FH-142   | 100 | 90  | 80  | 70  | 30  | 0   | 0   | 52.86 |
| FH-152   | 100 | 90  | 80  | 70  | 40  | 0   | 0   | 54.29 |
| FH-326   | 100 | 80  | 80  | 70  | 20  | 0   | 0   | 54.29 |
| Mubarak  | 100 | 80  | 70  | 50  | 20  | 0   | 0   | 45.71 |
| NIBGE-8  | 100 | 100 | 90  | 80  | 50  | 20  | 0   | 62.86 |
| NIBGE-9  | 100 | 100 | 90  | 70  | 40  | 10  | 0   | 58.57 |
| RH-668   | 100 | 100 | 90  | 70  | 40  | 10  | 0   | 58.57 |
| Mean     | 100 | 89.4| 80.6| 66.9| 35  | 5.63| 0   | 53.93 |

Alpha 0.05 Standard Error for Comparison = 1.4045

The results showed that the highest mean germination percentage under all PEG-6000 concentration was observed in the genotypes NIBGE-8 and DEEBEL having at par value of 62.86%, followed by NIBGE-9 and RH-668 with germination percentage of (58.57%), while the lowest germination percentage was shown by CYTO-313 and Mubarak with statistically at par value of 45.71%. From these results, it is concluded that all cotton genotypes can germinate up to 20% (0.491 MPa) concentration of PEG-6000.

Root length

The results showed that increment in root length was observed until PEG-6000 concentration of 10%, beyond that level root length was reduced leading to complete cessation at concentration level of 27%. Maximum root length (12.1 cm & 14.8 cm) was observed at -0.148 MPa under both growth stages (after 12th & 18th DAS) (Table-4). Under entire concentrations of PEG-6000, NIBGE-8 was the best performer with maximum root length (9.9 cm) succeeded by NIBGE-9 (9.19 cm) while Mubarak was the least performer with minimum root length (6.26 cm).

Shoot length

Analysis of shoot length data showed inverse relationship among PEG-6000 concentrations and the shoot length in all genotypes i.e., the shoot length decreased with rise of PEG-6000 concentration. On 18th day after sowing the mean shoot lengths under all concentrations of PEG-6000 (i.e. 0, 5, 10, 15, 20, 25 and 27%) were observed as 11.98, 10.32, 8.38, 5.64, 3.28, 0 and 0 cm respectively (Table-5). Maximum mean shoot length was observed in genotype NIBGE-8 (6.37cm) followed by NIBGE-9 (6.24 cm) and lowest shoot length was recorded in genotype Mubarak (4.67 cm). The shoot length was completely ceased at 25% concentration having osmotic potential of -0.735 MPa.
Table 4. Effect of different concentrations of PEG-6000 on root length (cm) at two growth stages

| Genotype | PEG-6000 concentration on 12th DAS | Mean | PEG-6000 concentration on 18th DAS | Mean |
|----------|------------------------------------|------|------------------------------------|------|
| BH-201   | 8.4 9.3 13.7 9 5 0.3 0 6.53 | 12.2 13.6 15.7 13 5.7 0.6 0 8.69 |
| CEMB-55 | 6.3 7.2 11.1 6.9 2.5 0 0 4.86 | 10.8 12.1 14.3 10.6 4 0 0 7.4 |
| CEMB-88 | 5.3 6.5 10.1 6.1 1.9 0.1 0 4.29 | 9.4 10.9 13 9.1 4.5 0.2 0 6.73 |
| CIM-602 | 8.2 9.1 13 8.6 4.5 0.2 0 6.23 | 12.1 13.5 15.5 13.1 6.7 0.5 0 8.77 |
| CIM-625 | 8 8.9 12.9 8.3 4.4 0.1 0 6.09 | 12 13.3 15.5 12.9 7.1 0.2 0 8.71 |
| CIM-632 | 7.5 8.2 12.2 8.1 4 0.1 0 5.73 | 11.4 12.8 15 12.6 6.3 0.2 0 8.33 |
| CYTO-179| 5.7 6.9 10.8 6.6 2.2 0 0 4.6 | 10.2 11.7 13.5 9.8 4.7 0 0 7.13 |
| CYTO-313| 5.9 7 11.1 6.7 2.4 0 0 4.73 | 10.3 11.7 13.8 10.1 5.4 0 0 7.33 |
| DEEBEL  | 5.5 6.8 10.5 6.3 2 0.1 0 4.46 | 9.9 11.4 13.4 9 4.5 0.3 0 6.99 |
| FH-142  | 7.7 8.4 12.3 8.2 4 0 0 5.81 | 11.4 12.9 15.1 12.4 6.2 0 0 8.29 |
| FH-152  | 7.5 8.1 12 8.1 3.9 0 0 5.66 | 11.3 12.4 14.7 12 5.9 0 0 8.04 |
| FH-326  | 7.9 8.7 12.7 8.3 4.2 0 0 5.97 | 11.7 13.1 15.3 12.8 6.1 0 0 8.43 |
| Mubarak | 4.7 5.8 9.9 5.4 1.2 0 0 3.86 | 9.3 10.5 12.7 8.7 2.6 0 0 6.26 |
| NIBGE-8 | 8.7 9.9 14.3 9.4 5.4 0.4 0 6.87 | 12.9 14.4 17.1 14.1 9.5 1.3 0 9.9 |
| NIBGE-9 | 8.5 9.5 13.9 9.1 5 0.2 0 6.6 | 12.5 14 16.5 13.6 8.6 0.9 0 9.44 |
| RH-668  | 8.2 9.2 13.4 8.7 4.9 0.2 0 6.37 | 12.3 13.8 15.8 13.3 8.4 0.7 0 9.19 |
| Mean    | 7.13 8.09 12.1 7.74 3.6 0.11 0 5.54 | 11.2 12.6 14.8 11.7 6.01 0.31 0 8.1 |

Alpha 0.05 Standard Error for Comparison = 0.0381 LSD = 0.1304

Root to shoot ratio
The result from data analysis of root to shoot ratio revealed that ratio was increasing with increase of PEG-6000 concentration. The maximum value of this ratio on 12th DAS and 18th DAS was observed at concentration levels of 20% and 15% respectively. There was a decreasing trend in root to shoot ratio on 18th DAS at 20% concentration level while increasing trend at 15% concentration level. However, on 18th DAS highest mean ratios were recorded for the genotypes NBGE-8 followed by RH-668 while minimum ratios were observed for cultivar BH-201. It is evident from results that maximum root to shoot ratio was recorded at 20% PEG-6000 concentration on 12th DAS followed by 10% concentration and minimum ratios were recorded at 25%-27% concentrations. Highest ratios on 18th DAS were recorded at 15% concentration followed by 20% concentration while least ratios are recorded at 25-27% concentration due to ceasing of root or shoot growth at these potentials (Table 5).
Table-6. Effect of different concentrations of PEG-6000 on cotton root to shoot ratio at different growth stages

| Genotype  | PEG-6000 concentration on 12\textsuperscript{th} day after sowing | PEG-6000 concentration on 18\textsuperscript{th} day after sowing |
|-----------|---------------------------------------------------------------|---------------------------------------------------------------|
|           | 0\%  5\%  10\%  15\%  20\%  25\%  27\%  Mean         | 0\%  5\%  10\%  15\%  20\%  25\%  27\%  Mean         |
| BH-201    | 0.8  0.9  1.7  3.1  0  0         | 1.11          |
| CEMB-55   | 0.7  0.8  1.6  1.5  2.8  0  0         | 1.06          |
| CEMB-88   | 0.6  0.9  1.9  1.6  2.7  0  0         | 1.1           |
| CIM-602   | 0.8  0.9  1.6  1.1  2.5  0  0         | 0.99          |
| CIM-625   | 0.8  0.9  1.6  1.3  2.9  0  0         | 1.07          |
| CIM-632   | 0.8  0.9  1.6  1.3  3.1  0  0         | 1.1           |
| CYTO-179  | 0.7  0.8  1.7  1.6  2.4  0  0         | 1.03          |
| CYTO-313  | 0.7  0.8  1.7  1.6  2.4  0  0         | 1.03          |
| DEEBEL    | 0.6  0.8  1.6  1.5  2.5  0  0         | 1.1           |
| FH-142    | 0.8  0.9  1.7  1.4  3.7  0  0         | 1.21          |
| FH-152    | 0.8  0.8  1.7  1.4  3.9  0  0         | 1.23          |
| FH-326    | 0.8  0.9  1.7  1.4  3  0  0          | 1.11          |
| Mubarak   | 0.6  0.9  2.3  1.5  2.4  0  0         | 1.1           |
| NIBGE-8   | 0.7  1  1.7  1.1  2.7  0  0         | 1.03          |
| NIBGE-9   | 0.7  0.9  1.7  1.2  3.3  0  0         | 1.11          |
| RH-668    | 0.8  0.9  1.8  1.4  3.5  0  0         | 1.2           |
| Mean      | 0.73  0.88  1.73  1.39  2.93  0  0     | 1.09          |
| Alpha 0.05 | Standard Error for Comparison = 0.0229 | LSD = 0.0785 |

Root length index

Analysis of data on root length revealed that root length index at both growth stages was highest at osmotic potential of -0.148 MPa (10\% PEG-6000 concentration) followed by -0.05 MPa (5\% PEG-6000 concentration) while the least was recorded at -0.846 MPa (27\% PEG-6000 concentration). Maximum mean root length index was recorded for genotype NIBGE-8 followed by NIBGE-9 while minimum root length index was recorded for genotype Mubarak at both developmental stages as well as at all concentrations of PEG-6000. At all levels of PEG-6000 (0.0, -0.05, -0.148, -0.295, -0.491, -0.735 and -0.846 MPa) mean root length index recorded on 12\textsuperscript{th} DAS were 712.5, 726.81, 980.81, 521.62, 130.5, 1.18 and 0 while 1123.12, 1132.12, 1197.5, 797.62, 219.18, 3.62 and 0, respectively were recorded on 18\textsuperscript{th} DAS (Table-7).

Shoot vigor index

The results of data analysis on mean shoot vigor index indicated that highest shoot vigor index was observed at both growth stages in NIBGE-8 followed by NIBGE-9 and least was recorded in Mubarak. At PEG-6000 concentrations 0, 5, 10, 15, 20, 25 and 27\% mean values recorded for shoot vigor index on 12\textsuperscript{th} DAS were 971.87, 812.12, 570.25, 388.5, 43.56, 0, and 0, respectively while at 18\textsuperscript{th} DAS recorded mean values for shoot vigor index were 1198.12, 924.12, 677.37, 378.5, 105.75, 0, and 0, respectively (Table-8).
Muhammad Riaz Gondal et al.

Table-7. Effect of different concentrations of PEG-6000 on cotton root length index at different growth stages

| Genotype | PEG-6000 concentration on 12th DAS | PEG-6000 concentration on 18th DAS |
|----------|-----------------------------------|-----------------------------------|
|          | 0% 5% 10% 15% 20% 25% 27% Mean   | 0% 5% 10% 15% 20% 25% 27% Mean   |
| BH-201   | 840 837 1096 630 200 3 0 515 1220 1224 1256 910 228 6 0 692 |
| CEMB-55  | 630 576 888 483 100 0 0 382 1080 968 1144 742 160 0 0 585 |
| CEMB-88  | 530 585 808 427 76 1 0 347 940 981 1040 637 180 2 0 540 |
| CIM-602  | 820 728 910 516 135 0 0 444 1210 1080 1085 786 201 0 0 623 |
| CIM-625  | 800 712 1032 498 88 0 0 447 1200 1064 1240 774 142 0 0 631 |
| CIM-632  | 750 738 976 567 160 1 0 456 1140 1152 1200 882 280 2 0 665 |
| CYTO-179 | 570 621 864 396 44 0 0 356 1020 1053 1080 588 94 0 0 548 |
| CYTO-313 | 590 560 777 335 48 0 0 330 1030 936 966 505 108 0 0 506 |
| DEEBEL   | 550 680 945 504 100 2 0 397 990 1140 1206 752 225 6 0 617 |
| FH-142   | 770 756 984 574 123 0 0 458 1140 1161 1208 868 186 0 0 652 |
| FH-152   | 750 729 960 567 156 0 0 452 1130 1116 1176 840 236 0 0 643 |
| FH-326   | 790 783 1016 581 168 0 0 477 1170 1179 1224 896 260 0 0 676 |
| Mubarak  | 470 464 693 270 24 0 0 274 930 840 889 435 52 0 0 449 |
| NIBGE-8  | 870 990 1287 752 270 8 0 597 1290 1440 1539 1128 475 26 0 843 |
| NIBGE-9  | 850 950 1251 637 200 2 0 556 1250 1400 1485 1088 344 9 0 797 |
| RH-668   | 820 920 1206 609 196 2 0 536 1230 1380 1422 931 336 7 0 758 |
| Mean     | 713 727 981 522 131 1.19 0 439 1123 1132 1198 798 219 3.63 0 639 |

Table-8. Effect of different concentrations of PEG-6000 on cotton shoot vigor index at different growth stages

| Genotype | PEG-6000 concentration on 12th DAS | PEG-6000 concentration on 18th DAS |
|----------|-----------------------------------|-----------------------------------|
|          | 0% 5% 10% 15% 20% 25% 27% Mean   | 0% 5% 10% 15% 20% 25% 27% Mean   |
| BH-201   | 1090 891 640 504 64 40 0 456 1270 981 720 455 156 0 0 512 |
| CEMB-55  | 900 696 552 315 36 40 0 357 1160 800 648 354 92 0 0 436 |
| CEMB-88  | 830 621 408 273 28 40 0 309 1090 855 592 315 76 0 0 418 |
| CIM-602  | 1050 792 574 462 54 0 0 419 1250 872 623 372 105 0 0 460 |
| CIM-625  | 1000 752 632 396 30 40 0 401 1230 856 712 366 68 0 0 462 |
| CIM-632  | 960 819 600 427 52 40 0 408 1200 945 688 399 120 0 0 479 |
| CYTO-179 | 870 738 512 246 18 40 0 341 1140 882 608 288 40 0 0 423 |
| CYTO-313 | 890 672 462 210 20 0 0 322 1160 808 588 275 54 0 0 412 |
| DEEBEL   | 850 810 576 328 40 0 0 372 1120 970 376 100 0 0 462 |
| FH-142   | 980 882 576 413 33 30 0 412 1220 945 704 420 102 0 0 484 |
| FH-152   | 970 873 568 399 40 40 0 407 1200 918 680 399 124 0 0 474 |
| FH-326   | 990 882 592 427 56 0 0 421 1210 918 664 378 112 0 0 469 |
| Mubarak  | 790 536 308 175 10 0 0 260 1050 736 497 220 30 0 0 362 |
| NIBGE-8  | 1190 1040 747 696 100 0 0 539 1330 1130 828 536 205 0 0 576 |
| NIBGE-9  | 1150 1010 720 511 60 0 0 493 1300 1110 819 455 160 0 0 549 |
| RH-668   | 1040 980 657 434 56 0 0 452 1240 1060 801 448 148 0 0 528 |
| Mean     | 972 812 570 389 43.6 0 0 398 1198 924 677 379 106 0 0 469 |

Discussion

The current research focused to investigate the drought tolerance in various genotypes of cotton. The data collection and analysis on various plant growth parameters comprised on germination percentage, root and shoot lengths, root to shoot ratio and vigor indices. These parameters were statistically analyzed, and explored thoroughly in comparison with each other and among their interactions with a wide range of

Asian J Agric & Biol. 2021(2).
Asian J Agric & Biol. 2021(2).

Muhammad Riaz Gondal et al.

Osmotic potentials. It was found that the PEG-6000 concentration showed inverse relationship with germination percentage i.e., as concentration increases germination decreases. Drop in germination percentage was due to water stress, which alters the cell function and growth. Xue-yan et al., (2008) found that cellular extension and carbohydrates wall production highly altered and inhibited due to water stress. Water stress ultimately decreases cell enlargement due to turgescence reduction (Shalhevet et al., 1995). The concentrations of 25-27% creates fatal osmotic potential for germination upon which germination stops (Sidari et al., 2008; Khodarahmpour, 2011; Babu et al., 2014; Megha and Mummigatti, 2017). Tsaliki et al. (2019) and Jatoi et al. (2014) also revealed that sprouting of cotton genotypes decreased under increased drought intensity with application of PEG-6000. Lesser PEG applied greater will be germination as adverse effect upon germination is dependent upon the proportion of drought intensity and duration in addition to PEG-6000 used.

It was observed that enlargement in the root length continued until PEG-6000 concentration reached at the level of 10%. Sakthivelu et al. (2008); Khodarahmpour (2011) and Jatoi et al. (2014) also reported decrease in root length under various water deficit conditions. This can be due to the reason that under osmotic stress plant separately execute additional photosynthesis for the enlargement and development of root instead of shoots. It facilitates plant to acquire moisture through deep penetration into the soil whereas reduced shoot size decreases transpiration rate (Tonin et al., 2000; Maruti and Katageri, 2015; Megha and Mummigatti, 2017). Long roots play important role in provision of water to plant by extracting water from the deep zone of soil as long roots are found to withdraw more water per unit length of root from moist ground and withdrawal of water reduces with the reduction of soil water potential (Landjeva et al., 2008 and Babu et al., 2014).

Shortening of shoot length could be due to the fact that under drought stress, plant tends to get moisture from the deep zone of soil, for which root size, number of roots, mass of root and adjacent roots became large and expanded which causes ultimate reduction in shoot length. These results are in complete agreement with the findings of Landjeva et al.,(2008); Sakthivelu et al. (2008); Khodarahmpour (2011); Babu et al. (2014) and Megha and Mummigatti (2017) who observed that shoot biomass decreases due to increase in root length volume, weight and lateral roots in search of moisture from deep soil layers. Declined length of shoot decreases transpiration rate due to decrease in surface area for water loss (Babu et al., 2014). Xue-yan et al. (2008) discovered that evaluation and selection of cotton genotypes can be carried out easily and rapidly for drought tolerance by the modification of osmotic conditions by means of PEG-6000. He subjected some cotton genotypes to artificial drought stress conditions for 12 hours by utilizing different concentrations of PEG at sprouting-, seedling-, cotyledon- and leaf formation stages. He observed varied amounts of osmotic stress tolerance and found that shoot development as well as 3-6 leaves formation phases were very crucial with respect to osmotic stress tolerance. Higher ratios might be due to ultimate increment in mass of roots, which enabled the plant roots to extract more water due to increased photosynthetic activity of plant for the development of higher root biomass. Lower shoot length and biomass assisted in the prevention of higher water losses by decreasing transpiration rate per unit area of shoot. They may change to maintain existence under osmotic stress conditions instead of having contribution in yield (Khodarahmpour, 2011; Babu et al., 2014 and Megha and Mummigatti, 2017). Meneses et al. (2011) reported that osmotic potential below -0.4 MPa have drastic effects upon seed viability and seedling vigor. Likewise earlier studies; on cotton by Michel and Kaufmann (1973), on cowpea by Ogbonnaya et al. (2003) and on wheat by Landjeva et al. (2008) revealed that genotypes, which were tolerant to drought stress circumstances attains higher root to shoot ratio as compared to the susceptible cultivars. Megha and Mummigatti, (2017) narrated that root length index decreases as osmotic potential increases by using PEG-6000 in susceptible genotypes but shoot vigor index showed inverse relationship with PEG-6000 concentration. The reduction in shoot vigor index is probably due to lengthy root and shoot; smaller number of leaves and reduced seedling length. Xue-yan et al. (2008) also reported the similar results.

Conclusion

From the current investigation, it is concluded that various cotton varieties showed different behavior against stress. Some varieties had significant tolerance against drought stress generated by PEG-6000 at some levels. Water deficiency highly effects the survival of
Muhammad Riaz Gondal et al.

seed and seedling development at different osmotic conditions. On basis of findings of this study, it is concluded that genotype NIBGE-8 was highly osmotic stress tolerant whereas cultivar Mubarak was highly sensitive to water stress. Furthermore, the results of the study revealed significance of PEG-6000 as synthetic stress inducer for quick evaluation and screening of drought tolerant cotton genotypes that can play key role in cotton breeding activities.

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Contribution of Authors

Gondal MR: Planned and managed the study, supervised data collection and analysis
Saleem MY: Conceived idea, designed research methodology, data collected and first draft write up
Rizvi SA: Interpreted data and final editing & approval of manuscript
Riaz A: Literature review and manuscript write up
Naseem W & Muhammad G: Literature review, data analysis and interpretation
Hayat S: Research execution and data collection
Iqbal M: Data analysis, final editing & approval of manuscript