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SCREENING OF COTTON (*Gossypium hirsutum* L.) GENOTYPES FOR DROUGHT TOLERANCE AT SEEDLING STAGE USING SLANTING GLASS TECHNIQUE IN THE NORTHERN GUINEA SAVANAH ECOLOGICAL ZONE OF NIGERIA

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is an important fibre crop in the world. The productivity of cotton is affected by several factors, of which drought have been identified as an important factor. Identifying drought-tolerant cotton genotypes is important hence, this study was undertaken to evaluate the performance of 12 cotton genotypes of cotton, viz: SAMCOT (8-13), CL-07, LINE 17, LINE 18, LINE 30, MA-1 and MA-15 using slanting glass plate with polyethylene glycol (PEG-6000) at 0, 10, and 20 g/L concentrations. The treatments were arranged in a 3 x 12 factorial experiment in a completely randomized design of three replicates. Sterilized seeds of the 12 cotton genotypes were arranged on the top portion of a filter paper and were inserted into a polyethene envelope containing prepared polyethylene glycol (PEG) solution at respective varying concentrations. Data were collected after three weeks of initiation for germination percentage, shoot length, shoot fresh weight, seedling vigour index, root fresh weight and root length. Highly significant ($P \leq 0.01$) % reduced germination was observed for shoot length, shoot fresh weight, seedling vigour, root length and root fresh weight as the PEG concentration increases, in which 20g/L PEG recorded least values in all the measured traits. Among the genotypes, LINE 30, SAMCOT 11 and SAMCOT 8 recorded the highest values for most of the traits. Therefore, these genotypes exhibit higher tolerance to PEG stimulated drought, hence suggesting production and confirmation for their tolerance to drought under field condition for adoption in mass production

Keywords: Cotton; drought; genotypes; seeds and PEG

INTRODUCTION

Cotton (*G. hirsutum* L.) is a leading non-food agricultural and industrial fibre crop grown in more than 80 countries (Dutt *et al.,* 2004). About 39.05 million metric tonnes of cotton are produced globally from 28.95 million hectares with an average productivity of 1.35 metric tonnes per hectare. India is the largest producer, with about 12.74 million metric tonnes from 10.85 million hectares with an average productivity of 1.06 metric tonnes and yield of about 0.98 metric tonnes per hectare on an area of 0.28 million hectares (FAOSTAT, 2018). Nigeria is ranked 29th in the world. The average world yield is 765kg/Ha while the Nigerian yield is 190kg/Ha on an area of 270 thousand hectares. The world average yield is higher than that obtained in Nigeria and this gap may be due to several factors such as biotic and abiotic stress like diseases, heat stress, drought stress, etc. (USDA, 2019). One of the major abiotic stresses affecting plant productivity is water stress due to drought which limits crop growth and productivity (Anjum *et al.,* 2011). Water availability and quality affect the growth and physiological processes of all plants as water is the primary component of actively growing plants ranging from 70-90% of plant fresh mass (Gardner and Gardner, 1983). Due to its predominant role in plant nutrient transport, chemical and enzymatic reactions, cell expansion and transpiration, water stresses result in anatomical and morphological alterations as well as changes in physiological and biochemical processes which affect the functions of plants (Megha and Mummigatti, 2017). Drought is regarded as a critical factor in cotton growing parts of the world because it adversely influences the yield of fibre and lint quality (Maqbool, 2009). Drought stress significantly reduces seed cotton production and productivity by affecting many agronomic traits like reduction in plant height, above-ground fresh weight, size and number of bolls per plant, seed cotton yield etc. (Malik *et al.,* 2006). Drought stress minimizes nutrient uptake and distribution within the body of plants, resulting in a reduction in growth and yield in cotton (Zhang *et al.,* 2017). Drought stress is an important threat among abiotic stresses, which has drastically affected normal growth of cotton plant (Hafeez *et al*., 2019). Drought stress causes stomata closure, which leads to decreased CO_2 intake, affecting the rate of photosynthesis and consequently reduced growth and yield (Chaves *et al*., 2009).

Polyethylene glycol (PEG) is a chemical commonly used for drought-stress related studies in plants (Turkhan *et al.,* 2005; Landjeva *et al.,* 2008). Polyethylene glycols with molecular masses of 6000 and above are non-penetrable and non-toxic osmotic substance which can be used to lower the water potential of the culture medium and it has been used to simulate drought stress in cultured plant tissues (Muhammad *et al.,* 2010; Khodarahmpour, 2011). Cotton has successfully been screened for drought tolerance using slanting glass technique (Babu *et al.,* 2014; Megha *et al*., 2017), this method is simple, fast and easily operated. The application of this technique in the screening for drought tolerance of Nigerian cotton lines will aid in the rapid screening and identification of drought-tolerant genotypes. Hence, this study was undertaken to evaluate 12 cotton genotypes for drought-tolerance using slanting glass technique to identify drought-tolerant cotton genotype(s).

MATERIALS AND METHODS

Study Area

The experiment was carried out in the Biotechnology Laboratory of the Department of Plant Science, Ahmadu Bello University Zaria located at Latitude $11^0$11'N and longitude $7^0$38'E in the northern Guinea savannah ecological zone of Nigeria.

Experimental Materials, Design and Procedures

The experimental materials used in the study comprised of 12 cotton genotypes (SAMCOT 8, SAMCOT 9, SAMCOT 10 SAMCOT 11, SAMCOT 12, SAMCOT 13, CL-07, LINE 17, LINE 18, LINE 30, MA-1 and MA-15) and three stress levels (0, 10g/L and

20g/L PEG). These were arranged in a 3 x 12 factorial experiment made up of 36 treatment combinations which were arranged in a completely randomized design and replicated three times. Rectangular glass plates of 3 mm thickness measuring 24 x 20 cm were used in the experiment. One glass plate was used per treatment. The glass plates were covered with fine quality filter paper sheets from top to bottom. Six healthy delinted seeds from each of the 12 cotton genotypes were surface sterilized with 80% ethanol for 3 minutes, after which 25% (v/v) Sodium hypochloride (commercial bleach) was added with 2 drops of tween20 for 20 minutes with occasional stirring under the laminar hood. The seeds were then rinsed thrice with sterile distilled water. The seeds were further placed on a filter paper lined on a glass plate arranged at 3 cm spacing. The seeds were then covered with a small strip of filter paper and placed in a slanting position. Clips were used to hold the assemblage of the glass plates to avoid displacement of seeds in the slanting position. Two millilitres (2 mL) PEG solution was added onto the bridge of the filter paper to each of the glass plate to facilitate adherence of seeds firmly to the filter paper. The glass plates were inserted into a polythene envelope designed to fit the glass plates. Two hundred and fifty millilitres (250 mL) of varying concentrations of PEG 6000 were added separately into the respective polythene envelope containing the cotton seeds in the slanting glass plate. Seedlings were germinated under room temperature for a period of 3 weeks. Fresh PEG solutions (50 mL) were added at regular intervals of three days to maintain the level of solution (Babu *et al.*, 2014).

Data Collection and Analysis

Data were collected on germination percentage (%), shoot length (cm), shoot fresh weight (g), seedling vigour index, root length (cm) and root fresh weight (g). Germination was recorded as percentage from three weeks of initiation by counting the seedlings that emerged from PEG-6000 solutions. Shoot length was measured from the medium contact point to the tip of the pant using a metre rule. Shoot fresh weight was measured by cutting the shoot from the seedling and weighed using a weighing balance. Seedling vigour was calculated by adding the values of root and shoot lengths and multiplied by germination percentage (%). Seedling vigour index = (root length + shoot length) × germination (Babu *et al*., 2014). Root lengths of seedlings were measured from the collar region to the tip of the longest root by using a meter rule. Roots were separated from the seedlings and weighed by using a weighing balance for root fresh weight. Data recorded were subjected to analysis of variance (ANOVA) using factorial ANOVA procedure of SAS 9.0 (SAS Institute Inc. 2002). Means were separated using Least Significant Difference (LSD).

RESULTS

Effect of PEG Concentration and Genotypes on Germination and Growth in Cotton

Highly significant differences ($P \leq 0.01$) were observed among the PEG concentrations for all the traits measured (Table 1). The highest (85%) germination percentage was observed in the control, while the least (55%) was observed in medium supplemented with 20g/L PEG. The control recorded the longest (9.69 cm) shoot length and highest (0.47g) shoot fresh weight while, the shortest shoot length (6.11 cm) and least shoot fresh weight (0.30g) were observed in the medium supplemented with 20g/L of PEG-6000. Similarly, the highest (2373) seedling vigour index was observed in the control, while the least (821) seedling vigour index was observed in medium with 20g/L PEG. Furthermore, longest and highest root length and root fresh weight (17.41cm and 0.09g) were recorded in the control while, shortest and least root length and root fresh weight (8.50cm and 0.06g) were respectively recorded in medium supplemented with 20g/L PEG.

Highly significant differences ($P \leq 0.01$) were observed among the genotypes for all the traits measured (Table 1), SAMCOT 11 recorded the highest germination percentage (86%), followed by LINE 18, LINE 30, SAMCOT 9 and CL-07 with germination percentages of 83%, 83%, 81% and 78%, respectively while SAMCOT 13 recorded the least (42%) germination percentage (Table 1).

Table 1: Effect of PEG 6000 and Lines on shoot and root traits of cotton

Treatments (PEG)	GP (%)	SHL (cm)	SHFWT (g)	SVI	RL (cm)	RFWT (g)
0	85.42[a]	9.69[a]	0.47[a]	2373.44[a]	17.41[a]	0.09[a]
10	68.06[b]	7.77[b]	0.38[b]	383.88[b]	11.94[b]	0.07[a]
20	55.21[c]	6.11[c]	0.30[c]	820.76[c]	8.50[c]	0.06[a]
Mean	69.56	7.86	0.45	1526.03	12.62	0.08
SE±	0.84	0.06	0.004	15.68	0.04	0.01
LSD	2.41	0.17	0.01	45.03	0.14	0.03
			Genotypes			
SAMCOT 8	72.22[d]	8.83[a]	0.53[ab]	1967.00[c]	16.14[c]	0.08[bcd]
SAMCOT 9	80.56[bc]	7.42[de]	0.32[f]	1661.00[e]	12.55[f]	0.15[a]
SAMCOT 10	63.89[e]	8.42[b]	0.47[cd]	1327.00[f]	11.55[g]	0.11[ab]
SAMCOT 11	86.11[a]	8.70[ab]	0.46[cd]	2153.00[b]	15.17[d]	0.10[abc]
SAMCOT 12	72.22[d]	7.25[e]	0.44d[e]	1324.00[f]	10.62[i]	0.05[bcd]
SAMCOT 13	41.67[f]	5.37[f]	0.36[f]	660.90[i]	10.22[j]	0.03[d]
CL-07	77.78[c]	7.70[cd]	0.33[f]	1758.00[d]	14.63[e]	0.07b[cd]
LINES 17	62.50[e]	7.63[d]	0.41[e]	1187.00[g]	11.05[h]	0.06[bcd]
LINES 18	83.33[ab]	7.73[cd]	0.48[c]	2078.00[b]	16.64[b]	0.08[bcd]
LINES 30	83.33[ab]	8.79[a]	0.48[c]	2280.00[a]	17.13[a]	0.08[bcd]
MA-1	66.67[e]	7.98[c]	0.52[b]	941.40[h]	5.63[k]	0.04[cd]
MA-15	44.44[f]	8.50[ab]	0.56[a]	974.30[h]	10.07[j]	0.07[bcd]
P x G	**	**	**	**	**	NS
Mean	69.56	7.86	0.45	1526.03	12.62	0.08
SE±	1.675	0.120	0.002	31.366	0.091	0.022
LSD	4.81	0.35	0.02	90.05	0.29	0.06
CV (%)	5.9	3.8	6.5	5.0	1.9	72.1

Mean with the same letter (s) in the same column are not significantly different at $P \leq 0.05$ level of significance using LSD. ** Highly significant. GP= Germination percentage, SHL= Shoot length, SHFWT= Shoot fresh weight, SVI= Seedling vigour index, RL=Root length, RFWT=Root fresh weight

The longest shoot length was observed in SAMCOT 8 followed by LINE 30, SAMCOT 11, MA-15, SAMCOT 10, LINE 18, CL-07, LINE 17 and SAMCOT 9 with shoot length of 8.83cm, 8.79cm, 8.70cm, 8.50cm, 8.42cm, 7.73cm, 7.70cm, 7.63cm and 7.42cm respectively while, SAMCOT 13 (5.37cm) recorded the least. MA-15, SAMCOT 8, MA-1, LINE 30 and LINE 18 recorded the highest shoot fresh weight of 0.56g, 0.53g, 0.52g 0.48g and 0.48g respectively while, SAMCOT 9 recorded the least shoot fresh weight (0.32g).

Among the 12 cotton genotypes, LINE 30, recorded highest seedling vigour index (2280) followed by SAMCOT 11, LINE 18, SAMCOT 8, CL-07 and SAMCOT 9 with seedling vigour index of 2153, 2078, 1967, 1758 and 1661 respectively while SAMCOT 13 recorded the least seeding vigour index value (661). LINE 30 (17.13cm) recorded the longest root length followed by LINE 18, SAMCOT 8, SAMCOT 11, CL-07, SAMCOT 9 and SAMCOT 11 with, 16.64cm, 16.14cm, 15.17cm, 14.63cm, 12.55cm and 11.55cm values respectively while, MA-1 recorded the shortest (5.63cm) root length. SAMCOT 9 and SAMCOT 10 had the highest (0.15g and 0.11g) values for root fresh weight while SAMCOT 13 recorded the least root length (0.03g).

Interaction Effect of PEG Concentration and Cotton Genotypes

The interaction effects between PEG concentrations and genotypes were highly significant ($P \leq 0.01$) for all the measured traits (Table 1) The highest germination percentage was recorded in the control treatment by SAMCOT 8 (100%) followed by the interaction of the control with SAMCOT 11, LINE 18, LINE 30 and SAMCOT 9 (100%, 100%, 100% and 91.66% respectively) while SAMCOT 13 recorded the least (50%) (Fig 1a). In 20g/L of PEG, SAMCOT 11, SAMCOT 9, SAMCOT 12, CL-07, LINE 18 and LINE have germination percentage of 75, 67, 67, 67, 67 and 67 respectively), while MA-1 recorded the least (17%) germination (Fig 1a).

SAMCOT 11 recorded the maximum shoot length followed by SAMCOT 8 and MA-15 with 12.50cm, 11.65cm and 11.50cm values respectively while, SAMCOT 13 recorded the minimum shoot length (6.50cm) in the control (Fig. 1b). At 20g/L of PEG, SAMCOT 10 recorded the maximum followed by LINE 18, LINE 17, SAMCOT 8 and SAMCOT 9 with 7.45cm, 7.23cm, 7.13cm, 6.83cm, and 6.48 values respectively, while SAMCOT 13 recorded the minimum (3.40cm) shoot length.

MA1 and MA-15 recorded the maximum shoot fresh weight (0.73g and 0.72g), while SAMCOT 13 recorded the minimum (0.36g) shoot fresh weight in the control (Fig. 1c) when the medium was supplemented with 20g/L PEG, SAMCOT 8 and CL-07 recorded the maximum and minimum (0.51g and 0.26g) shoot fresh weight, respectively.

In the control, SAMCOT 8 recorded maximum seeding vigour index followed by SAMCOT 11 and LINE 30 with 3630, 3605 and 3525 respectively, while SAMCOT 13 recorded the minimum (981) (Fig. 1d). In 20g/L of PEG, LINE 18 recorded maximum followed by CL-07, SAMCOT 11 and SAMCOT 12 with 1363, 1237, 954 and 943, respectively while MA-15 recorded the minimum (193) seedling vigour index.

In the control treatment, SAMCOT 8 recorded longest root length followed by LINE 30 and SAMCOT 11 with 24.65cm, 24.60cm and 23.55cm respectively while, MA-1 recorded the shortest (6.83cm) root length (Fig. 1e) Meanwhile, LINE 18 recorded the longest followed by CL-07 and SAMCOT 8 with 13.23cm, 13.10cm and 11,30cm respectively, while MA-1 recorded the shortest (4.65cm) root length in 20g/L PEG.

SAMCOT 10 and SAMCOT 11 recorded the maximum (0.156g and 0.15g), while SAMCOT 13 the minimum (0.04g) root fresh weight (Fig. 1f). In the case of 20g/L of PEG, SAMCOT 9 and SAMCOT 13 recorded the maximum and minimum (0.29g and 0.02g), respectively.

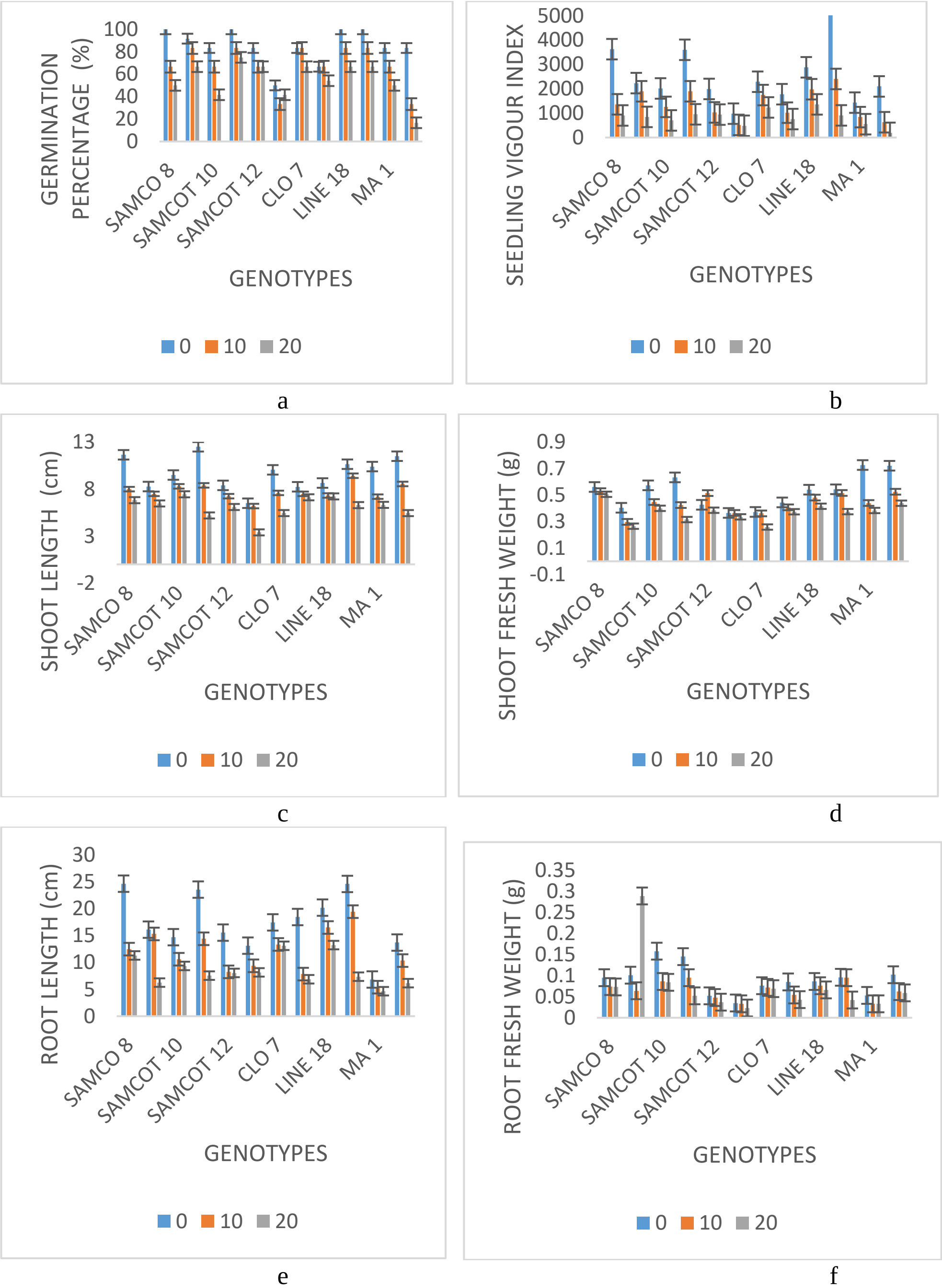

Fig. 1: Effect of PEG concentration x Genotype interaction on: a) germination percentage of cotton (%); b) seedling vigour index of cotton; c) shoot length of cotton (cm); d) shoot fresh weight of cotton (g); e) root length of cotton (cm); and f) root fresh weight of cotton (g)

DISCUSSION

Germination percentage decreases as the PEG concentration increases from 0 to 20g/L. In their findings, Megha *et al.* (2017) reported a decrease in seed germination percentage as the PEG 6000 concentration increases from 0% to 20% in cotton using slanting glass plate technique. Decreased germination might be due to increased osmotic stress which results into water deficit that damaged cellular machinery. This also agrees with the findings of Babu *et al*. (2014) who reported 25% to 27% PEG concentrations as the lethal water potential for germination of cotton seeds. Results from the study also show that shoot length, shoot fresh weight and seedling vigour index decreases with the increase in PEG concentration from 0 to 20g/L. Similarly, Babu *et al.* (2014) and Megha *et al*. (2017) observed shoot length, shoot fresh weight and seedling vigour index decreased with the increase in PEG-6000 concentrations from 0% to 20%. Under moisture stress condition plants exhibit increase in root length, root volume, root weight and lateral roots for water absorption from deeper surfaces, which results in decreased decrease in shoot biomass (Megha *et al*., 2017). This could be the reason for the reduction in shoot biomass with increasing PEG concentration as observed in our study.

Among the cotton genotypes SAMCOT 8, SAMCOT 11, Line 30 and MA-15 recorded the highest values for most of the shoot traits as compared with values of the other cotton genotypes, which may be attributed to their inherent differences. Genotypic variation for shoot traits has also been reported in tomatoes, sunflower and cactus cultures from using similar technique (Aazami *et al*., 2010; Turhan and Baser, 2004; Mengesha *et al*., 2016). Megha *et al*. (2017) screened twenty-one cotton genotypes and reported that genotypes that well under various PEG concentrations were considered to be tolerance to osmotic stress. Zhang *et al*. (2007) also observed varietal difference while screening 13 cotton varieties with PEG 6000 at different concentrations. The interaction effect was found to be significant for most of the shoot traits with SAMCOT 8, SAMCOT 9, SAMCOT 11, SAMCOT 12, CL-07, LINE 17 and LINE 18 recording the maximum values under 20g/L PEG.

Root length decreases with an increase in PEG concentration from 0 to 20g/L with the control recording the highest value while medium with 20g/L of PEG recorded the least. This is contrary to the work of Babu *et al*. (2014) who reported that root length was increased with the increasing PEG-6000 concentrations up to 10% PEG-6000 and it declined thereafter. The increased root length might be due to the partitioning of more photosynthates for root growth rather than the shoots, for the absorption of more water from deeper surfaces under water stress. The decreased shoot organs help in the reduction of transpiration which results in water loss from the shoot surfaces (Babu *et al*., 2014). The observed differences among the cotton genotypes could be attributed to genotypic differences. Water stress-sensitive genotypes show more reduction in root growth, whereas drought stress tolerant genotypes appeared to be relatively less stressed under moisture stress conditions in cotton (Hassan *et al*., 2015). Significant PEG x Lines interaction effect was observed for root length with SAMCOT 8, CL-07 and LINE 18 recording the longest root length, while SAMCOT 9 recorded higher root fresh weight under 20g/L. Iftikhar *et al*. (2019) observed that root traits such as root length, fresh root weight, and dry root weight contribute more to drought tolerance as compared to the shoot related traits.

CONCLUSION

Based on the findings of this study, PEG at 20g/L affected all the traits indicating its suitability for stimulating droughts conditions for screening cotton using slanting glass plate. Among the genotypes evaluated, SAMCOT 8, SAMCOT 9, CL-07 and LINE 18 recorded the maximum values for most of the traits under 20g/L PEG induced stress, suggesting these genotypes to be drought tolerant compared to the other genotypes. However, findings from the current study can be confirmed by field study evaluation for update.

REFERENCES

Aazami, M.A., Torabi, M. and Jalili, E. (2010). *In vitro* response of promising tomato genotypes for tolerance to osmotic stress. *American Journal of Biotechnology,* 9(26): 4014-4017.

Anjum, A.S., Xie, X., Wang, L., Saleem, M.F., Man, C. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6(9): 2026-2032.

Babu, A.G., Patil, B.C. and Pawar, K.N. (2014). Evaluation of Cotton Genotypes for Drought Tolerance Using PEG-6000 Water Stress by Slanting Glass Plate Technique. *The Bioscan*, 9(2): 1419-1424.

Chaves, M.M., Flexas, J. and Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Journal of Anatomy and. Botany*, 103: 551-560.

Dutt, Y., Wang, X.D., Zhu, Y.G. and Li, Y.Y. (2004). Breeding for high yield and fibre quality in coloured cotton. *Journal of Plant Breeding*, 123:145-151.

FAOSTAT (2018). Food and Agriculture Organization Statistics *Online Internet: http://apps.fao.org/agriculture* (accessed September, 2019).

Gardner, W.R. and Gardner, H.R. (1983). Principles of water management under drought conditions. *Journal of Agricultural Water Management*, 7:143-155.

Hafeez, M.N., Ahmad, S., Mamoon, U.R., Rashid, M., Ali, A., Salman, S., Bakhsh, A., Mahpara, S., Kamaran, S., Ramzan, I., Waseem, M., Ali, Q. and Rashid, B. (2019). An overview of enhancing drought tolerance in cotton through manipulating stress resistance genes. *Applied Ecology and Environmental Research*, 17(3): 7003-7025.

Hassan, M.U., Maqsood, M., Majid, S.A. and Ranjha, A.M. (2015). Rooting pattern and nitrogen use efficiency in cotton (*Gossypium hirsutum* L.) under moisture stress conditions. *Journal of Animal and Plant Science*, 25: 1429-1440.

Iftikhar, M.S., Talha, G.M., Shahzad, R., Jameel, S., Aleem, M. and Iqbal, M. Z. (2019). Early response of cotton (*Gossypium hirsutum* L.) genotype against drought stress. *International Journal of Biosciences,* 14 (2): 537-544.

Khodarahmpour, Z. (2011). Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrid. *African Journal of Biotechnology*, 10(79): 18222-18227.

Landjeva, S., Neumann, K., Lohwasser, U. and Borner, M. (2008). Molecular marking of genomic regions associated with wheat seedlings growth under osmotic stress. *Journal of Plant Biology,* 52:259-266.

Malik T. A., Sanaullah and S. Malik. (2006). Genetic linkage studies of drought tolerant and Agronomic traits in cotton. *Pakistan Journal of Botany*, 38(5): 1613-1619.

Maqbool, A. (2009). Search for drought tolerant genes through different display. PhD Thesis, the University of Punjah, Lahore, India.

Megha, B.R. and Mummigatti, U.V. (2017). Evaluation of Hirsutum Cotton Genotypes for Drought Tolerance under Different Osmotic Potential and Filed Capacities. *International Journal of Bioresource and Stress Management*, 8(2): 299-308.

Megha, B.R., Mummigatti, U.V., Chimmad V.P. and Aladakatti, Y.R. (2017). Evaluation of Hirsutum Cotton Genotypes for Water Stress using Peg-6000 by Slanting Glass Plate Technique. *International Journal of Pure and Applied Bioscience*, 5(2): 740-750.

Mengesha, B., Mekbib, F. and Abraha, E. (2016). *In vitro* screening of Cactus (*Opuntia ficus-indicia* [(L.Mill] Genotypes for Drought Tolerance. *American Journal of Plant Science*, 7: 1741-1758.

Muhammad, H., Khan, S.A., Shinwari, Z.K., Khan, A.L., Ahmad, N. and In-Jung, L. (2010). Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. *Pakistan Journal of Botany*, 42(2): 977-986.

SAS Institute Inc. (2002). *SAS/STAT User's Guide*, version 6, 4th Ed. SAS Inc. Cary, NC, USA.

Turkhan, I., Bor, M., Ozdemir, F. and Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant tepary beans (Phaseolus aculitifolius Gray and drought sensitive common beans (*Phaseolus vulgaris* L.) subjected to poylethylene glycol mediated water stress. *Journal of Plant Science*, 168: 223-231.

Turhan, H. and Baser, I. (2004). *In vitro in vivo* water stress in sunflower *(Helianthus annus* L.) *Helia*, 27(40): 227-236.

USDA (2019). United State Department of Agriculture. Human Development Report Database. Newyork .http//hdr.undp.org/en/statistics/. Accessed 8 May 2020.

Zhang, X.Y., Liu, C., Wang, J., Li, F. and Ye, W. (2007). Drought tolerance evaluation of cotton with PEG water stress method. *Journal of Cotton Science*, 19(3): 205-209.

Zhang, H., Khan, A. and Tan, D.K. (2017). Rational water and nitrogen management improves root growth, increases yield and maintains water use efficiency of cotton under mulch drip irrigation. *Front Plant Science*, 8:912.

APPENDIX

APPENDIX 1: Effect of PEG concentration on Seedling Growth in Cotton I) Seedlings in slanting glass plate II a) Control b) Seedling on 10g/LPEG c) Seedling on 20g/L PEG