

EVALUATION OF COTTON GENOTYPES SEEDLINGS FOR DROUGHT STRESS TOLERANCE

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Abstract: The present study indicated that the expressivity of some of the genes was changed due to the simulated drought conditions. The root increases in length in response to drought, thus utilising more photosynthates changing the sink and ultimately reducing the shoot growth. The shift due to drought indicated that the growth and morphological development of root system are under genetic control but may be modified by environmental influences. To screen out drought tolerant and drought susceptible genotypes based on morphological seedling traits such as root characters and root / shoot length ratio. After screening, three drought tolerant genotypes, namely BH-124,149-F and DPL-26 and three drought susceptible genotypes i.e. CIM-446, FH-945 and VH-28 were identified, which may be used in further hybridization programmes to develop drought tolerance varieties and hybrids.

Keywords: cotton, drought, root length, shoot length, hybrids

Introduction

Cotton (Gossypium hirsutum L.) is grown for fiber and seed throughout the world, however, drought stress results in significant yield reductions. Cotton fiber consists of hairs growing from the epidermis of the seed. The fiber is woven into fabrics or combined with other fibers. Cotton replaced wool during the 19th century. It is an important natural fiber. Although synthetic fibers are commonly used in the textile industry, natural fibres are still preferred in making garments. On average, two-thirds of the harvested crop comprises cottonseedcottonseed, an important oil source, used in cooking as vegetable oils. Low grade oils are utilized in soap and lubricants manufacturing. Residual seed cake is a valuable protein concentrate used for livestock feed. So cotton has manifold benefit both for human and livestock in the form of fiber, food, fuel and feed. It is grown in tropical and subtropical regions of more than 80 countries worldwide (Singh, 2004; Ali et al., 2017; Ahamd et al., 2012). Cotton crop has a lot of potential to boost the country's economy. Cotton farmers require high yielding cotton cultivars that are agronomically adaptive and physiologically efficient bom under well-watered and drought conditions. Water is available on the earth' surface in huge quantity yet is the most limiting factor to crop production. Cotton and other plants must maintain a balance between the water supply stored in the soil system and the evaporative demand for carrying outgrowth. Shortage of water has been a major limitation in the yield of cotton also due to high temperatures (Ali et al., 2013, 2016, 2014ab; Hafeez et al., 2021; Javed et al. 2012).

Materials and methods

A set of 40 genotypes of {Gossypium hirsntum L.) were grown in greenhouse using a normal water supply and limited water conditions at the seedling stage, during the year November 2006 in controlled light and temperature to provide the recommended environment for germination will be maintained at 30°C during daytime and 25°C during nighttime by using heaters. The plants were exposed to sunlight and supplemented with artificial light to provide a photoperiod of 14 hours. Seeds of the genotypes were sown in polythene bags (25 X 15 cm) filled with silt @ 1250 grams per bag. The experiment was conducted using three replications. The treatments arranged following completely randomized design. After germination, all plants watered and fertilized till the first true leaf's development. The plants in control condition watered regularly to keep the soil at field capacity (100% soil water saturation), while in other unit water stress imposed by withholding water. The effect of water stress monitored visually and with soil moisture meter (HH2 Theta Probe Type, Delta-T device, Cambridge, England). At the initial wilting stage, when soil had 14 to 16% soil moisture content, the stressed plants watered enough to relieve the sign of wilting but insufficient to reach field capacity. The experiment lasted 45 days until the third main stem



leaf was fully expanded. The control and stressed plants measured for the following screening traits.

Fresh root length

The detached root of each of the three plants of each entry was measured for length with the help of measuring tape in cm, averaged the data for analysis.

Fresh shoot length

Shoot of each of three plants from each repeat was measured length wise in cm, and calculate their average.

Fresh shoot weight

Detached shoot of each of the three plant of each genotype was weighed with the help of electronic balance, three plant averages was used for the analysis.

Dry Shoot Weight

Detached shoots of each of the three repeat plants were put in the oven (Gen. Lab. Ltd. Windedness, England) at a controlled temperature of 70°C for 48 hours for complete drying and shoot was weighed with the help of electronic balance and their averages were calculated.

Tissue moisture percentage

At the end of the experiment, tissue moisture percentage was calculated with the help of the following formula.

Tissue moisture % = Shoot fresh weight -Shoot dry weight /shoot fresh weight.

Root/shoot ratio

The root/ shoot ratio was determined at the end of the experiment using the following formula:

Root/shoot ratio = Root length / Shoot length x 100. **Results**

Root Length

As regarding the mean values of root length measured under normal water condition experiment (Table 2), it is evident that 40 genotypes differed from each other and ranged from 14.03 cm for FH-950 (No. 12) to 5.43 cm for FVH-53 (No. 27). Under drought conditions, root lengths were markedly reduced and ranged from 12.63 cm for FH-950 (No.12) to 3.73 cm for CIM-496 (No. 26). Data on root lengths revealed that accessions have differing responses to the two moisture conditions. Genotypes FH-950 (No.12) and FH-925 (No. 13) had the tallest root lengths under control conditions measuring 14.03 and 14.00 cm, respectively, whilst FH-950 (No. 12), gave maximum length under drought conditions. In contrast, genotypes FVH-53 (No. 27), CIM-496 (No.26) and BH-147 (No. 25) had shorter root lengths under control measuring 5.43, 7.80 and 7.97 cm, respectively, whilst under stress conditions, CIM-496 (No.26) developed shortest root length. Based on least reduction in root length under drought conditions, the cotton genotypes 149-F (4.15% loss), MNH-552 (4.27% loss), BH-124 (4.39%loss) and S-14(4.70%loss), these genotypes are rated as drought tolerant. In contrast, the genotypes VH-28 (No. 5),

FH-945 (No. 23) and CIM-446 (No. 14) showed varied responses to the two moisture conditions, e.g root length of these genotypes was 9.07, 9.40 and 13.0 cm respectively under normal condition, whilst under drought condition these measured 5.40 cm, 6.00 cm and 8.40 cm respectively. Due to drastic reduction in root lengths in drought conditions, these genotypes may be rated drought susceptible.

Shoot Length

Table 2 indicated the mean performance of various cotton genotypes ({Gossypium hirsutum L.) for shoot length under normal and drought conditions. In this parameter, the genotypes appeared to respond differently to non-stressed and stressed conditions. The shoot length under normal conditions ranged from 28.33 cm for OKRA 659 (No. 35) to 13.87 cm forBH-147 (No.25). Shoot lengths under drought conditions were markedly reduced and varied from 22.13 cm for MNH -147 (No.34) to 7.03 cm for CIM-496 (No. 26) and similar differences were recorded among other genotypes. Cotton genotypes OKRA 659 (No.35), CIM-446 (No. 14) and MNI1-147 (No. 34) had the longest shoot length under control, each measuring 28.33, 27.50 and 26.47 cm, against shoot length of BH-147 which was only 13.87 cm. Under water stress, MNH-147 (No. 34) and MNH-129 (No.38), had the longest shoot length i.e 22.13 and 19.57 cm, against shoot length of CIM-496, which was only 7.03 cm (No.26) the genotypes.

Shoot fresh weight

The means of various genotypes for the expression of fresh shoot weight (Table 3) under normal conditions ranged from 6.31gm to 1.84gm. While in drought conditions, it ranged from 4.91gm to 0.94gm. The genotype 4-F(No.8), MNH-147(No.34), MNH-129(No.38)under normal condition. Exhibited the highest means, 6.31gm, 6.00gm, 5.98gm and S-12(No.21), MNH-786(NO.1), COKER 4601(NO.33)had the lowest shoot fresh weight, 1.84gm,2.09gm, 2.20gm. The genotype MNH-147(No.34) followed by CIM-446(No.14)and 199-F(No.39) under drought condition had the highest shoot fresh weight 4.91gm, 3.50gm, 3.48gm and CIM-496(No.26), DPL-26(No.19), NIAB-999(No.9) had the least shoot fresh weight 0.94gm, 1.26gm, 1.31gm, respectively.

Shoot dry weight

Table 3 indicated the means of all forty genotype of cotton (*Gossypium hirsutum* L.) for shoot dry weight. This table showed that the mean ranges from 0.99gm to 0.32gm and 0.85gm to 0.16gm, under normal and drought conditions respectably. Under normal moisture condition experiment, the genotypes MNIM47(No.34), CIM-446(No.14), VH-141(No.16) showed the highest mean (0.99gm,0.91gm, 0.83gm and was significantly different from the genotype

CIM-496(No.26), CIM-448(No.32), S-14(No.20) which had the lowest (0.32gm, 0.38gm, 0.39 gm mean while under drought condition experiment, the genotype MNH-147(No.34), CIM-446(No.14), MNH-129(No.38)showed the highest mean value (0.85gm, 0.79gm, 0.72gm and the genotype 149-F(No.7), CIM-496(No.26), CIM-448(No.32), had the lowest value for shoot dry weight, 0.16gm, 0.19gm, 0.20gm.

Tissue moisture percentage

The mean highest tissue moisture percentage (Table 3) was produced by genotype MN11-129 (No.38)(92.02gm), followed bv BH-124(No.22)(91.90gm), NIAB-KARISHMA (No.10) (90.85gm) and the lowest number was observed in cotton genotype MNH-786(No.l)(71.30gm), FH-900(No.1 1)(80.18gm), BH-160(No.15)(80.90gm) under normal moisture condition experiment. Whereas under drought condition, the cotton 149-F genotype (No.7)(92.01 gm)followed bv BH-124(No.22)(91.54gm) and DPL-26(No.19)(90.42gm) had the highest tissue moisture percentage while the genotype VH-28(No.5)(74.77gm) followed FHby

945(No.23)(76.79gm) and CIM-446(No.14) (77.40gm) had the lowest tissue moisture percentage. **Root/shoot ratio**

Mean highest root/shoot ratios (Table 3) were produced by genotypes BH-118(No.29), BH-124(No.22) and MNH-552(No.3) ranging 0.75gm,0.73gm and 0.71gm respectively whilst lower ratios were observed in genotypes FVH-53(No.27), FH-945(No.23) and OKRA 659(No.35) having values 0.38, 0.41 and 0.43gm, under normal water condition. Under drought stress condition, the differential responses of genotypes to water stress for root/shoot ratio is obvious from their values (Table 3). Genotype 149-F (No.7) is the most tolerant with ratio of 1.14 followed by BH-124(No.32) and DPL-26(No.19) with values of 1.00 and 0.97 respectively, In contrast, the most sensitive genotype is VH-28(No.5) with ratio of 0.40 and is closely followed by FH-945(No.23) and CIM-446(No.l4) with values of 0.43 and 0.44 respectively. Under water stress condition experiment, cotton genotypes both maximum and minimum root /shoot length ratio were marked based on their mean values. The genotypes are as given in Table 4.

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Table1 Mean squares and coefficient of variation (C.V '	%) for various cotton seedling traits in greenhouse
under normal and drought conditions	

Character	Character Under Norm			mal Under Drought						
	Replication (DF2)	Genotype (DF39)	Error (DF 78)	C.V%	RepMeat ion (DF2)	Genotype (DF39)	Error (DF 78)	CV%		
Root length	0.058	12.759**	0.036	1.70	0.072	14.815**	0.147	4.03		
Shoot length	0.085	44.982**	0.297	2.80	0.240	31.532**	0.229	353		
Shoot fresh weight	0.039	4.633**	0.017	3.23	0.007	2.112**	0.002	3.84		
Shoot dry weight	0.001	0.075**	0.000	3.16	0.001	0.083**	0.001	6.22		
Root /shoot ratio	0.000	0.021**	0.000	3.23	0.003	0.091**	0.001	5.18		
Tissue moisture percentage	0.292	48.828**	0.378	0.72	0.627	47.646**	0.605	0.92		

Table 2	Means	of seedling traits	of cotton	genotypes	under	normal	and
drought con	dition in	glass house.					

Sr.#	Genotypes	Root Length (cm)		Shoot Length (cm)		Root / Shoot Ratio	
		Normal	Drought	Normal	Drought	Normal	Drought
1	MNH-786	10.90	8.87	16.43	12.00	0.66	0.74
2	MNH-93	10.03	8.23	17.53	11.47	0.57	0.72
3	MNH-552	12.50	11.97	17.70	12.77	0.71	0.94
4	MNH-554	12.10	10.12	21.77	15.63	0.56	0.65
5	VH-28	9.07	5.40	18.17	13.40	0.50	0.40
6	CIM-240	11.10	10.37	16.67	12.17	0.67	0.85
7	149-F	12.87	12.33	21.30	10.80	0.60	1.14
8	4F	9.00	6.50	19.80	14.07	0.45	0.46
9	NIAB-999	11.80	10.87	19.40	11.40	0.61	0.95
10	NIAB-KAR1SHMA	12.37	11.07	18.97	12.77	0.65	0.87

11	FH-900	12.97	11.03	23.80	19.00	0.55	0.58
12	FH-950	14.03	12.63	23.20	16.10	0.60	0.79
13	FH-925	14.00	12.57	22.03	13.20	0.64	0.95
14	CIM-446	13.00	8.40	27.50	18.93	0.47	0.44
15	BH-160	12.67	10.73	19.53	14.63	0.65	0.73
16	VH-141	12.97	12.17	23.57	15.97	0.55	0.76
17	VH-144	13.60	12.00	26.37	15.67	0.52	0.77
18	CIM-1100	12.03	10.80	20.90	17.10	0.58	0.63
19	DPL-26	9.03	8.23	14.53	8.47	0.62	0.97
20	S-14	9.93	9.47	17.67	12.43	0.56	0.76
21	S-12	8.40	7.90	13.90	9.10	0.60	0.87
22	BH-124	10.63	10.17	14.63	10.17	0.73	1.00
23	FH-945	9.40	6.00	22.80	14.03	0.41	0.43
24	CIM-473	11.00	10.20	17.97	15.97	0.61	0.64
25	BH-147	7.97	7.23	13.87	12.60	0.57	0.57
26	CIM-496	7.80	3.73	16.33	7.03	0.48	0.53
27	FVH-53	5.43	5.00	14.30	9.43	0.38	0.53
28	FH-901	8.03	7.40	14.33	10.67	0.56	0.70
29	BH-118	12.97	12.00	17.20	13.77	0.75	0.87
30	B1I-36	8.13	7.30	16.70	10.97	0.49	0.66
31	BH-116	10.87	9.07	17.97	10.40	0.61	0.87
32	CIM-448	11.20	8.90	18.57	11.03	0.60	0.81
33	COKER 4601	10.57	8.90	18.40	13.03	0.57	0.68
34	MNH-147	13.97	11.37	26.47	22.13	0.53	0.51
35	OKRA 659	12.07	9.90	28.33	16.37	0.43	0.61
36	DIXI-KING	10.93	9.87	17.87	12.83	0.61	0.77
37	VH-54	12.70	9.03	21.30	17.70	0.60	0.51
38	MNH-129	13.23	12.10	23.63	19.57	0.56	0.62
39	199-F	13.00	11.13	22.67	14.80	0.57	
40	SLH-257	10.90	9.70	15.97	12.90	0.68	0.75

 Table 3.
 Means of seedling traits of cotton genotypes under normal and drought condition in glass house.

Sr.#	Genotype	Shoot Fres (£ni)	Shoot Fresh Weight (£ni)		Shoot Dry Weight (fim)		Tissue Moisture %	
		Normal	Drought	Normal	Drought	Normal	Drought	
1	MNH-786	2.09	1.71	0.60	0.29	71.30	82.87	
2	MNH-93	3.80	1.95	0.47	0.31	87.67	84.09	
3	MNH-552	5.67	2.93	0.63	0.48	88.85	83.69	
4	MNH-554	3.49	2.40	0.63	0.44	82.09	81.80	
5	VH-28	4.30	2.28	0.43	0.31	84.09	74.77	
6	C1M-240	3.81	2.54	0.52	0.31	86.40	87.76	
7	149-F	3.39	1.96	0.41	0.16	87.99	92.01	
8	4F	6.31	2.69	0.59	0.28	90.63	89.71	
9	NIAB-999	3.67	1.31	0.41	0.22	88.89	83.60	
10	NIAB-	4.39	2.10	0.40	0.30	90.85	85.89	
	KARISHMA							
11	FH-900	2.41	1.26	0.48	0.21	80.18	83.11	
12	FH-950	4.22	2.82	0.40	0.31	90.54	88.85	
13	FH-925	5.13	2.17	0.48	0.31	90.64	85.87	
14	CIM-446	5.98	3.50	0.91	0.79	84.72	77.40	

15	BH-160	3.78	2.65	0.72	0.52	80.90	80.49
16	VH-141	5.59	2.68	0.83	0.36	85.13	86.43
17	VH-144	5.83	2.92	0.77	0.48	86.82	83.43
18	CIM-1100	4.91	2.45	0.62	0.32	87.45	86.85
19	DPL-26	2.41	1.26	0.42	0.27	86.54	90.42
20	S-14	2.46	1.39	0.39	0.25	83.99	82.02
21	S-12	1.84	1.80	0.32	0.23	82.76	87.00
22	BH-124	3.80	1.95	0.49	0.29	91.90	91.54
23	OFH-945	4.80	2.40	0.53	0.42	86.53	76.79
24	CIM-473	4.00	1.92	0.42	0.31	89.44	84.13
25	BH-147	3.97	2.33	0.61	0.47	84.64	79.83
26	CIM-496	2.49	0.94	0.32	0.19	87.14	80.30
27	FVH-53	3.03	1.96	0.52	0.33	82.45	83.40
28	FH-901	4.30	2.28	0.70	0.34	83.67	84.91
29	BH-118	3.17	2.07	0.50	0.28	84.15	86.37
30	BH-36	3.10	2.12	0.57	0.28	81.58	86.65
31	BH-116	4.26	1.40	0.43	0.28	89.89	79.92
32	CIM-448	3.39	1.96	0.38	0.20	89.27	86.73
33	COKER4601	2.20	1.93	0.38	0.19	82.85	89.96
34	MNH-147	6.00	4.91	0.99	0.85	83.56	82.62
35	OKRA 659	3.99	2.41	0.57	0.51	85.70	79.00
36	DIXI-K1NG	4.81	2.41	0.52	0.38	89.27	84.40
37	VH-54	5.02	3.21	0.73	0.63	85.50	80.51
38	MNH-129	5.98	3.50	0.49	0.72	92.02	83.90
39	199-F	4.34	3.48	0.75	0.65	82.63	81.27
40	SLH-257	3.08	2.50	0.57	0.43	81.58	82.76

Table 4. Cotton Genotype with Their Stress Status

Genotype	Root/Short Ratio	Remarks
149-F	1.14	Drought tolerant
BH-124	1.00	Drought tolerant
DPL-26	0.97	Drought tolerant
VH-28	0. 40	Drought Susceptible
FH-945	0.43	Drought Susceptible
CIM-446	0.44	Drought Susceptible

Discussions

Potential genotypes have been selected reliably based on seedling traits (Basal et al., 2005; Longenberger et al, 2006). In addition, seedling traits have also been used to evaluate many genotypes for drought tolerance and play an important role in obtaining desirable crop stands. Hence the endeavours to increase cotton production have been made through developing improved seedling traits. .Several different seedling traits have been suggested as important relative to drought tolerance. These include lateral and tap root weight, lateral root is an important and reliable indicator of the response of drought tolerant varieties (Basel et al, 2005). Therefore this character was also examined at the seedling stage. The present investigations examined 45-day old seedlings of 40 accessions, grown under normal water

and drought stress conditions in the greenhouse for four morphological characters. This method distinguished tolerant and non -tolerant accessions and provided data to study the growth pattern of accessions with the least environmental influences. Previously, scientists had also studied the response of cotton genotypes to moisture stress under greenhouse conditions (Aaliya et al., 2016; Abbas et al., 2015, 2016; Puspito et al., 2015; Zafar et al., 2022). Many reports have been documented regarding growth and response of cotton genotypes to moisture stress environment under greenhouse conditions (Saranga et al, 2004) in a growth chamber (Genty et al., 1987; Nepomuceno et al., 1998), under field conditions in arid (Leidi et al, 1999) and humid environment. However, the present studies on screening for drought tolerance was conducted under greenhouse conditions.

The response of accessions to water stress conditions have been compared with those measured under nonstress conditions based on shoot length, root length, shoot fresh weight and shoot dry weight, root-toshoot ratio and tissue moisture percentage. Water stress tolerance cannot be attributed to a genotype because of its superiority for a single trait; therefore, many different parameters were required to be evaluated (Al-Hamdani and Barger, 2003). Root

growth is an important and reliable indicator of the response of drought-tolerant varieties (Ball *et al.*, 1994; Basel *et al*, 2005), and therefore this character was also examined at the seedling stage because root length is less sensitive than shoot length according to Malik *et al* (1979), McMichael and Quisenberry (1991).

Conflict of interest

The authors declared the absence of conflict of interest.

References

- Aaliya, K., Qamar, Z., Ahmad, N. I., Ali, Q., Munim, F. A., & Husnain, T. (2016). Transformation, evaluation of gtgene and multivariate genetic analysis for morpho-physiological and yield attributing traits in Zea mays. *Genetika*, 48(1), 423-433.
- Abbas, H. G., Mahmood, A., & Ali, Q. (2015). Genetic variability and correlation analysis for various yield traits of cotton (*Gossypium hirsutum* L.). *Journal of Agricultural Research*, 53(4), 481-491.
- Abbas, H. G., Mahmood, A., & Ali, Q. (2016). Zero tillage: a potential technology to improve cotton yield. *Genetika*, 48(2), 761-776.
- Ahmad, H. M., Ahsan, M., Ali, Q., & Javed, I. (2012). Genetic variability, heritability and correlation studies of various quantitative traits of *mung bean* (Vigna radiate L.) at different radiation levels. *International Research Journal of Microbiology*, 3(11), 352-362.
- Al-Hamdani, S.H. and T.W. Barger, 2003. Influence of water stress on selected physiological responses o three sorghum genotypes. *Italy J. Agron.*, 7:15-22.
- Ali, F., Ahsan, M., Ali, Q., & Kanwal, N. (2017). Phenotypic stability of Zea mays grain yield and its attributing traits under drought stress. *Frontiers in plant science*, 8, 1397.
- Ali, Q., Ahsan, M., Ali, F., Aslam, M., Khan, N. H., Munzoor, M., ... & Muhammad, S. (2013). Heritability, heterosis and heterobeltiosis studies for morphological traits of maize (Zea mays L.) seedlings. Advancements in Life Sciences, 1(1):52-63.
- Ali, Q., Ahsan, M., Kanwal, N., Ali, F., Ali, A., Ahmed, W., ... & Saleem, M. (2016). Screening for drought tolerance: comparison of maize hybrids under water deficit condition. Advancements in Life Sciences, 3(2), 51-58.
- Ali, Q., Ali, A., Ahsan, M., Nasir, I. A., Abbas, H. G., & Ashraf, M. A. (2014a). Line× Tester analysis for morpho-physiological traits of Zea mays L seedlings. *Advancements in Life sciences*, 1(4), 242-253.
- Ali, Q., Ali, A., Awan, M. F., Tariq, M., Ali, S., Samiullah, T. R., ... & Hussain, T. (2014b).

Combining ability analysis for various physiological, grain yield and quality traits of Zea mays L. *Life Sci J*, **11**(8s), 540-551.

- Ball, R.A., Oosterhuis, D.M. and Mauromoustakos, A. 1994. Growth dynamics of the cotton plant during water-deficit stress. Agronomy Journal, 86: 788-795.
- Basal, H., C.W. Smith, P.S. Thaxton, and J.K. Hemphill. 2005. Seedling drought tolerance in upland cotton. Crop sci. 45(2): 766-771.
- Genty, B., J.M. Briantais and J.B. Vieira da Silva. 1987. Effects of drought on primary photo synthetic processes of cotton leaves. Plant Physiology, 83: 360-364.
- Hafeez, M. N., Khan, M. A., Sarwar, B., Hassan, S., Ali, Q., Husnain, T., & Rashid, B. (2021). Mutant Gossypium universal stress protein-2 (GUSP-2) gene confers resistance to various abiotic stresses in *E. coli* BL-21 and CIM-496-*Gossypium hirsutum. Scientific Reports*, 11(1), 20466.
- Javed, I., Ahsan, M., Ahmad, H. M., & Ali, Q. (2016). Role of mutation breeding to improve Mungbean (Vigna radiata L. Wilczek) yield: An overview. *Nature Science*, **14**(1), 63-77
- Leidi, E.O., Lopez.J. Gorham and J.C. Gutierrez. 1999. Variation in carbon isotoj discrimination and other traits to drought tolerance in upland cotton cultiva: under dryland conditions. Field Crops Res. 61:109-123.
- Longenberger, P.S., C.W. Smith, P.S. Thaxton and B.L. McMichael. 2006. Developme of a screening method for drought tolerance in cotton seedlings. Crop Sci. 4: 2104-2110.
- Malik, R.S., J.S. Dhankar, and N.C. Turner. 1979. Influence of soil water deficits on re growth and cotton seedlings. Plant Soil 53:109-115.
- McMichael, B.L., and J.E. Quisenberry. 1991. Genetic variation for root-she relationships among cotton germplasm. Environ. Exp. Bot. 31:461-470.
- Nepo-muceno, A.L., D.M. Oosterhuis, and J.M. Stewart. 1998. Physiological response cotton leaves and roots to water deficit induced by polyethylene glycol. Envirc Exp.Bot, 40(1): 29-41.
- Puspito, A. N., Rao, A. Q., Hafeez, M. N., Iqbal, M. S., Bajwa, K. S., Ali, Q., ... & Husnain, T. (2015). Transformation and evaluation of Cry1Ac+ Cry2A and GTGene in *Gossypium hirsutum* L. *Frontiers in Plant Science*, 6, 943
- Saranga, Y., C.X. Jiang, R.J. Wright, D. Yakir and A.H. Paterson. 2004. Genetic dissection of cotton physiological responses to arid conditions and their interrelationships with productivity. Plant Cell and Environment, 27: 263-277,

- Singh, P. 2004. Cotton Breeding. 2nd ed. Kalyani Publishers, New Delhi: 1, 2, 6. 147, ,148, 149, and 162.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A biometrical approach. 3rd ed. WCB/McGraw Hill, Inc. New York, USA.



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Zafar, M. M., Mustafa, G., Shoukat, F., Idrees, A., Ali, A., Sharif, F., ... & Li, F. (2022). Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. *Scientific Reports*, **12**(1), 10878.