Pak. J. Agri. Sci., Vol. 59(3), 391-403;2022
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/22.39
http://www.pakjas.com.pk

Estimation of genetic effects and interrelationship of morpho-physiological and biochemical attributes for drought tolerance in upland cotton (*Gossypium hirsutum* L.)

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The knowledge about genetic effects controlling drought related parameters is necessary to understand the quantitative nature of the parameters responsible for drought tolerance mechanism. For instance, MNH-886 and MNH-988 were crossed with FH-114 and FH-Kehkshan to develop generations (F_1, F_2, BC_1 and BC_2), respectively. Parents and developed generations were planted in the field area using triplicate Randomized Complete Block Design under two environmental conditions *i.e*., normal and drought. Data were recorded for morpho-physiological and biochemical attributes. Mean performance of the traits and estimation of genetic effects revealed quantitative nature of the traits. The presence of dominance main effect in abundance along with epistatic interactions particularly additive × dominance and dominance × dominance in most of the traits suggested for delayed selection in later segregating generations. Additionally, low-moderate narrow-sense heritability also suggested the same. Interrelationship of physiological and biochemical attributes with yield related traits revealed desirable association that would be helpful at the time of selection. Thus, the findings could be used as potential material in further breeding approaches for development of drought tolerant cotton varieties.

Keywords: Generation mean analysis, gene action, correlation coefficient analysis, epistatic interaction, peroxidase activity, segregating populations.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important commercially grown fiber crop in Pakistan which is known as white gold because of its major impact on economy and foreign exchange reserves of a country (Khan *et al*., 2020). However, the current cotton production is below the cultivar's potential in Pakistan. It is due to the biotic and abiotic stresses (Anonymous, 2020-21). The primary factors responsible for reducing crop production are abiotic stresses and accounted more than 50% yield reduction in the world (Hassan *et al*., 2020).

Among abiotic stresses, drought is the main problem for limiting cotton production (Rosolem *et al*., 2019). It occurs due to low water precipitation, high temperature, high light intensity and salinity causing changes in morphological, biochemical, physiological and molecular traits in plants being drought is a multidimensional stress (Kapoor *et al*., 2020). It imposes a threat to effective growth and development of cotton crop (Fathi and Tari, 2016) by affecting various traits like plant height, stem dry weight, canopy and root development, node number, leaf dry weight and leaf area index (Loka *et al*., 2011). Physiological attributes like carboxylation efficiency, water potential of cotton leaves, rate of transpiration, stomatal conductance and photosynthesis are also affected (Kumar *et al*., 2001) ultimately reducing the production of the crop by effecting boll weight, seed cotton yield and bolls per plant (Malik *et al*., 2006).

Drought stress also causes non stomatal restrictions to photosynthesis (Parida *et al*., 2007). ROS (Reactive oxygen species), consisting of hypochlorous acid, hydrogen peroxide, hydroxyl radical, singlet oxygen, nitric oxide, anion radical, peroxyl radical and ozone is a major aspect causing changes in multiple metabolites like cellular particles, proteins, lipids and photosynthetic pigments (Mittler, 2002). The accretion of ROS is activated by low moisture content in the plant cells which costs physiological homeostasis (Fernandez-Ocana *et al*., 2011) and oxidative impairment at cellular level (Iannone *et al*., 2012). In response, the antioxidant defense components

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Imtiaz, M., A. Shakeel, A.I. Khan and M.S.N. Rehman. 2022. Estimation of genetic effects and interrelationship of morpho-physiological and biochemical attributes for drought tolerance in upland cotton (*Gossypium hirsutum* L.). Pakistan Journal of Agricultural Science. 58:391-403.

[Received 6 Jan 2022; 15 M\ay 2022; Published 27 Jun 2022]

viz., superoxide dismutase, peroxidase and catalase support plants to maintain balance between ROS scavenging agents and its production to overcome oxidative stress (Ali and Ashraf, 2011). Additionally, the accumulation of proline content facilitates antioxidants by controlling osmotic regulation eventually lessens the oxidative stress at cellular level (Kavi Kishor and Sreenivasulu, 2014). Hence, proline content shows a positive response to drought resistance (Ahmed *et al*., 2013). Various researchers have also studied different biochemical parameters *viz*., soluble sugars, proline contents, chlorophyll contents, peroxidase, catalase, hydrogen peroxide and superoxide activities, and reported these parameters as indicators for drought stress tolerance (Feng *et al*., 2011; Hasan *et al*., 2018; Jie *et al*., 2020).

The understanding about the drought tolerance mechanism in cotton crop is important because of its complex and quantitative nature depending on different morpho-physiological, biochemical and environmental factors. This makes it necessary to gather information about gene actions responsible for the improvement of drought tolerance in cotton plants (Munir *et al*., 2007). Therefore, the estimation of genetic effects using generation mean analysis is appropriate for complex traits controlling drought tolerance in plants (Zdravkovic *et al*., 2011). Moreover, it would help to develop drought tolerant cotton varieties by adopting suitable breeding approaches (Shakoor *et al*., 2010). In consideration to the above-mentioned facts, the objectives of the current research were (i) to study genetic architecture of morpho-physiological and biochemical attributes towards drought tolerance and (ii) to analyze association of physiological and biochemical attributes with yield related traits under drought stress conditions.

MATERIALS AND METHODS

Selection of Plant Material: The plant material was selected from screening experiment conducted under hydroponic conditions against drought stress at Wire House, department of Plant Breeding & Genetics, University of Agriculture Faisalabad, Pakistan (PBG-UAF). Thirty-seven cotton genotypes were collected from various institutes of Pakistan *viz*., National Institute of Biotechnology and Genetic Engineering, Center for Excellence in Molecular Biology, Central Cotton Research Institute, University of Agriculture Faisalabad, Islamia University of Bahawalpur, Nuclear Institute for Agriculture and Biology, Nuclear Institute of Agriculture and, Cotton Research Institutes. These cotton genotypes were evaluated under drought-imposed conditions on the base of physiological parameters. Two highly drought tolerant MNH-886 (Mahmood *et al*., 2021) and MNH-988 (Farooq *et al*., 2021) while two highly drought sensitive FH-114 (Ullah *et al*., 2019) and FH-Kehkshan (Majeed *et al*., 2019) cotton genotypes were selected from the screening experiment.

Development of Plant Material: Selected cotton genotypes (Parents) were crossed divergently to develop F_1 populations (MNH-886 × FH-114 and MNH-988 × FH-Kehkshan). It is due to the hypothesis of conducted analysis that the generations under study must be developed from divergent parents (Salmi *et al*., 2019) to study gene action under drought conditions.

To develop F_1 population, parental cotton seed was grown in earthen pots filled with loamy soil at green house (October, 2018). Green house facilities were maintained with temperature (28-30ºC on day time and 20-25ºC at night time), day length (16 hours approx.) and relative humidity (50-60%) for proper germination and growth of parental cotton seed. Contrasting parents were crossed to produce F_1 population seed. Subsequently, parental and F_1 population seed was sown in field area to develop segregating populations (F_2, BC_1 and BC_2) (May, 2019). At the end, six generations were developed from each cross combination (Fig. 1).

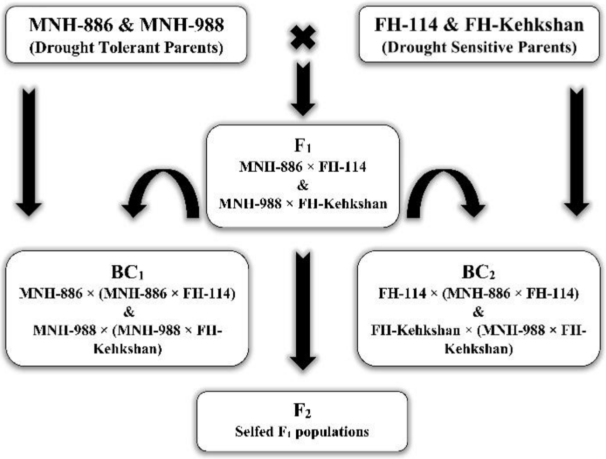


Figure 1. Development of generations of Cross 1 (MNH-886 × FH-114) and Cross 2 (MNH-988 × FH-Kehkshan).

Field Experiment: The experiment was conducted in the field area of PBG-UAF located at 31.4407° N, 73.0702° E (May, 2020). Developed populations of cross combinations were raised in Randomized Complete Block Design containing three replications in each trial under normal and drought conditions. Each cross combination along with developed populations were planted and analyzed separately. Parents and F_1 populations were planted in two rows in each replication while ten rows for backcrosses and fifteen rows for F_2 populations were planted because of their segregating nature. Standard distances were maintained in plantation of experiment for in-between plants (30cm) and in-between rows (75cm). All agronomical and cultural practices were performed according to cotton production technology. The trial conducted under normal conditions received 22 acre-

Table 1. Meteorological data during the cotton growing season 2020.

Parameters	Months, 2020							
	April	May	June	July	August	September	October	November
Rainfall (mm)	28.30	29.80	49.20	87.80	183.30	9.00	0.00	1.40
Mean Max. Temp. (°C)	34.50	40.00	40.30	39.64	38.00	37.38	36.32	27.85
Mean Min. Temp. (°C)	18.20	23.30	26.50	28.79	28.61	26.31	17.27	10.78
Mean Relative Humidity (%)	43.00	50.80	58.50	64.50	73.03	69.16	59.09	63.26

Source: Meteorological unit, department of Crop physiology, University of Agriculture Faisalabad, Pakistan.

inches of irrigational water whereas drought-imposed experiment was irrigated with 12 acre-inches of water. Moreover, 13.59 inches of rainfall received was also recorded during the conduction of experiment (Table 1).

Data Collection: Measurements for drought tolerance were taken during 3rd week of October when drought symptoms started appearing. Data were recorded from each population for traits under study. 10 plants were marked from each population for parents and F_1 population in each replication for data recording. However, 20 plants were chosen for backcrosses whereas 50 plants were marked for F_2 population. Physiological and biochemical attributes *viz*., stomatal size (SS), stomatal frequency (SF), peroxidase activity (POD), catalase activity (CAT), hydrogen peroxide (H_2O_2) and proline estimation (PRO) were recorded and analyzed under in vitro conditions while plant height (PH), bolls per plant (BP) and seed cotton yield (SCY) were measured in vivo. Plant height was measured in centimeters from soil surface to the apical bud of plant using measuring rod. It was suggested to measure plant height after physiological cutout, reflecting towards development of last bolls population *i.e.*, five nodes above white flower (Oosterhuis and Kerby, 2008). Number of bolls per plant were measured by collecting bolls in all the pickings from individual plant. It was then averaged for each population per replication. The total bolls picked up in all the pickings from a plant constitutes to seed cotton yield of a plant. It was weighed in grams and averaged to obtain seed cotton yield of a population per replication.

For stomatal size and frequency, fully expanded leaf samples were collected from selected plants in experiment. 1 inch wide and 2 inches long strips were cropped from the samples and preserved in 50-100ml plastic bottles filled with Alcohol fixative (Acetic acid + Ethanol). After 3-4 days, samples were prepared to be used for slide preparation. Slides were prepared by putting a leaf strip on a clean cloth and excessive liquid was removed from it. Then, nail varnish was applied on the abaxial surface of the leaf strip and left it for 3-4mins to dry. A piece of transparent tape was placed on a strip portion where nail varnish was applied. It was then gently pulled over from leaf strip that took out epidermal layer. This piece of tape was placed on glass slide to examine under microscope. This technique is known as impression method suggested by Wang and Clarke (1993). Stomatal frequency was measured in a microscopic field by observing under 10X objectives whereas stomatal size was measured under 40X objectives. Biochemical attributes were measured by collecting leaf samples from field experiment at harvesting stage, immediately transferred to laboratory in cool box and stored at -80 ºC. Measurement of peroxidase activity, catalase activity, hydrogen peroxide content and proline content were recorded following the suggested methods by Liu *et al*. (2009), Chance and Maehly (1955), Liu *et al*. (2010) and Bates *et al*. (1973), respectively.

Statistical Analysis: The variability among generations used in the study was tested using standard analysis of variance method, explained by Steel *et al*. (1997). Significant differences among generations led to check the genetic effects of various traits using a method known as generation mean analysis (Mather and Jinks, 1982). Generation means and variances were calculated from individual plants of each population (Parents, backcrosses, F_1 and F_2) per replication to conduct weighted least square analysis. Initially, simplest model (m) was used in the analysis. If the fitted model was significant on the base of chi-squared value then further models of increasing complexity would be fitted. Therefore, the best fitted model was chosen when chi-squared value

Table 2. Components of genetic effects for the weighted least squares analysis (Mather and Jinks, 1982).

Generations	Components of genetic effects					
	Coefficients of the mean (m)	Additive (d)	Dominance (h)	Additive × Additive (i)	Additive × Dominance (j)	Dominance × Dominance (l)
P_1	1	1.0	0.0	1.00	0.00	0.00
P_2	1	-1.0	0.0	1.00	0.00	0.00
F_1	1	0.0	1.0	0.00	0.00	1.00
F_2	1	0.0	0.5	0.00	0.00	0.25
BC_1	1	0.5	0.5	0.25	0.25	0.25
BC_2	1	-0.5	0.5	0.25	-0.25	0.25

remained non-significant with significant estimation of parameters. Components of genetic effects of generation mean in theoretical manner are given in Table 2. Narrow-sense heritability (h^2_{ns}) was estimated using a good fitted model's variance components from weighted least square analysis. Correlation studies among traits were also calculated from data of F_2 populations (Individual plants) using correlation coefficients (Dewey and Lu, 1959).

RESULTS

Genetic variability and mean performance of traits: Mean squares of populations of both cross combinations (MNH-886 × FH-114 and MNH-988 × FH-Kehkshan) for various traits including PH, BP, SCY, SS, SF, POD, CAT, H_2O_2 and PRO under both water conditions *i.e.*, normal and drought from variability analysis are presented in Table 3. Significant differences were observed among generations of cross combinations for all the parameters under both water treatments except peroxidase activity (POD) in Cross 2 (MNH-988 × FH-Kehkshan) under normal conditions. The differences among generations would lead to investigate genetic effects of traits and association among them using generation mean analysis and correlation coefficient analysis. The mean values of generations of both cross combinations for various traits under normal and drought conditions along with percent decrease or increase of traits under drought conditions are presented in Fig. 2 and 3.

Genetic effects controlling drought related traits: Generation mean analysis for generations of Cross 1 (MNH-886 × FH-114) and Cross 2 (MNH-988 × FH-Kehkshan) on the basis of various traits under normal (N) and drought (D) conditions along with narrow-sense heritability (h^2_{ns}) are given in Table 4.

Plant height (PH): In case of plant height, the model with four parameters (m, d, h, i) was good fit to data in Cross N1, D1 and N2 whereas four parameters (m, d, j, l) was better fit for Cross D2. The magnitude of dominance effect (h) among genetic effects was considerably high in Cross N1 and N2 whereas it was reduced in Cross D1 but missing in Cross D2. However, additive (d) type of gene action was increased in Cross D1 and D2. It reflects to the change of inheritance pattern from dominance to additive effect. For the epistasis, additive × additive (i) type of interaction was observed in Cross N1, D1 and N2 while additive × dominance (j) and dominance × dominance (l) type of interactions prevailed in Cross D2. The overall genic control under water stress regime was governed by additive and epistatic effects. Narrow-sense heritability of 0.27 was estimated in Cross N1 and D1 whereas it was estimated as 0.29 in Cross N2 and 0.31 in Cross D2.

Table 3. Mean squares of six generations of Cross 1 (MNH-886 × FH-114) and Cross 2 (MNH-988 × FH-Kehkshan) for morpho-physiological and biochemical attributes under normal and drought conditions.

Traits	Normal			Drought		
	Replication	Generations	Error	Replication	Generations	Error
			Cross 1			
PH	8.52	721.51**	86.92	13.95	207.75*	37.23
BP	83.01	285.48**	45.23	21.90	229.93**	20.40
SCY	185.12	1923.68*	469.70	18.20	965.26**	10.39
SS	1142.43	1260.16**	197.14	2268.01	1044.27**	153.90
SF	29.76	405.76**	35.54	91.80	781.72**	87.95
POD	0.17	7.64*	1.78	0.59	26.29**	1.06
CAT	1.53	333.04**	14.34	6.26	574.70**	14.63
H_2O_2	0.0003	0.0017*	0.0004	0.00029	0.00029**	0.00029
PRO	0.0064	0.0135**	0.0016	0.00320	0.03810**	0.00180
			Cross 2			
PH	1.37	49.20*	10.57	8.88	175.42**	13.22
BP	12.18	103.17**	14.28	7.22	120.06**	17.37
SCY	20.35	848.54**	50.91	10.78	1320.50**	83.01
SS	149.08	2581.41*	650.63	303.50	1495.69**	82.18
SF	646.00	345.34**	32.87	473.06	805.42**	75.87
POD	2.98	3.68^{NS}	3.21	0.82	3.00*	0.78
CAT	1.79	58.34**	4.39	8.78	112.73**	5.07
H_2O_2	0.0002	0.0013*	0.0003	0.00009	0.0048**	0.00015
PRO	0.0012	0.014**	0.0014	0.00180	0.0742**	0.00390

*, P < (0.05); **, P < (0.01) Where, PH; plant height (cm), BP; no. of bolls per plant, SCY; seed cotton yield (g), SS; stomatal size (µm), SF; stomatal frequency, POD; peroxidase activity (U/mg), CAT; catalase activity (U/mg), H_2O_2; hydrogen peroxide (µmole/g) and PRO; proline content (µmole/g).

Bolls per plant (BP): For bolls per plant, the models with five parameters (m, d, h, i, j) in Cross N1 and four parameters (m, d, h, l) in Cross D1 were good fit to data.

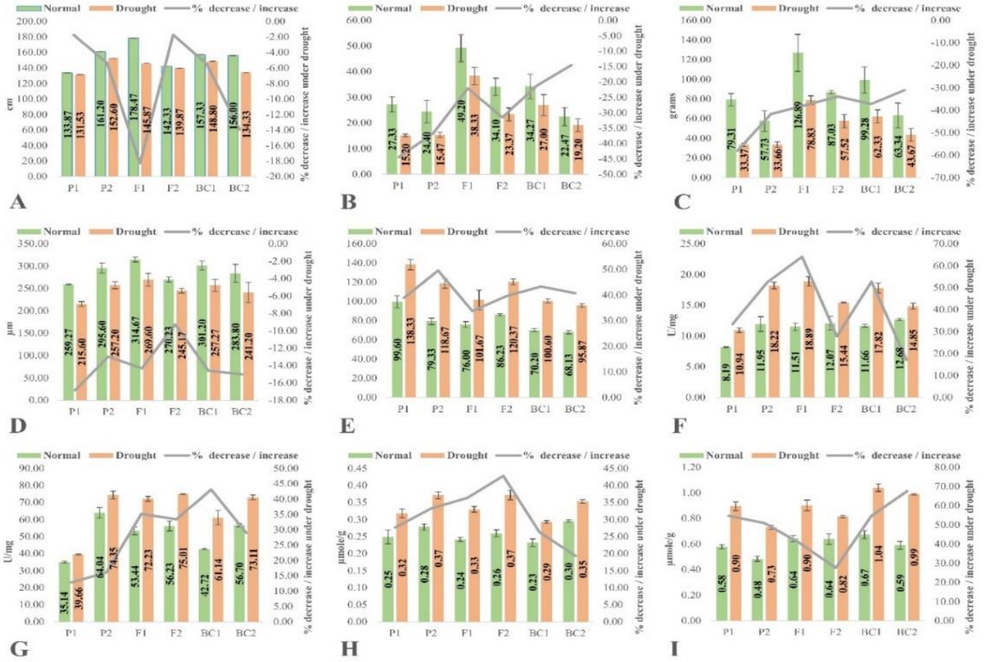
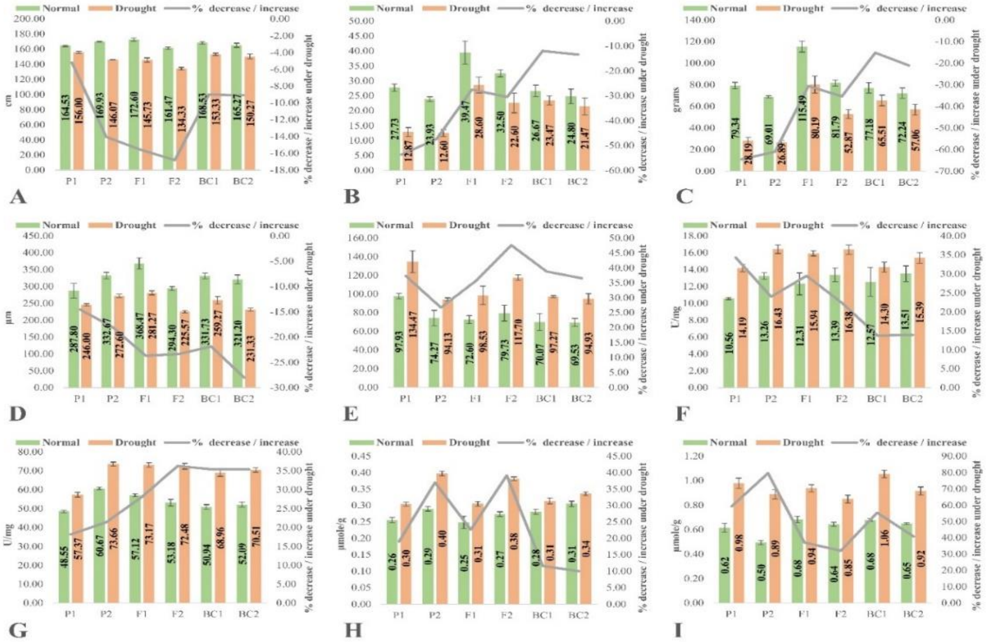


Figure 2. Mean performance of (A.) plant height, (B.) no. of bolls per plant, (C.) seed cotton yield, (D.) stomatal size, (E.) stomatal frequency, (F.) peroxidase activity, (G.) catalase activity, (H.) hydrogen peroxidase content and (I.) proline content under both water treatments (Normal and drought) along with percent decrease or increase due to drought stress in generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of Cross 1 (MNH-886 × FH-114). Bar above each column represents standard error.



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(I.) proline content under both water treatments (Normal and drought) along with percent decrease or increase due to drought stress in generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of Cross 2 (MNH-988 × FH-Kehkshan). Bar above each column represents standard error.

Table 4. Estimation of genetic effects in respect of various traits of Cross 1 (MNH-886 × FH-114) and Cross 2 (MNH-988 × FH-Kehkshan) under normal and drought conditions in the field.

Traits	Cross #	Genetic Effects						χ^2 (df)	h^2_{ns}
		(m) ± S.E.	(d) ± S.E.	(h) ± S.E.	(i) ± S.E.	(j) ± S.E.	(l) ± S.E.		
PH	N1	133.8±2.30	5.44±0.80	102.5±4.31	55.7±2.26	-	-	0.19(2)	0.27
	D1	91.4±1.70	12.02±1.08	11.7±2.96	7.8±2.17	-	-	3.17(2)	0.27
	N2	149.5±1.80	2.87±0.83	23.9±3.33	17.9±2.00	-	-	1.57(2)	0.29
	D2	142.5±0.58	5.23±0.96	-	-	4.03±3.31	4.18±1.83	1.49(2)	0.31
BP	N1	22.2±2.36	1.93±1.02	21.9±4.11	2.4±2.52	23.49±4.79	-	2.32(1)	0.25
	D1	15.6±0.69	1.48±0.63	7.2±2.85	-	-	15.45±3.47	2.20(2)	0.22
	N2	26.5±0.45	1.87±0.59	-	-	0.31±2.66	14.80±1.64	3.77(2)	0.26
	D2	16.3±1.67	0.14±0.58	12.9±2.76	3.4±1.75	4.33±2.63	-	1.20(1)	0.20
SCY	N1	66.4±2.31	11.72±2.41	41.1±4.67	-	49.89±11.22	-	2.48(2)	0.28
	D1	33.4±0.80	0.14±0.85	45.3±1.73	-	37.70±5.42	-	3.65(2)	0.20
	N2	70.1±0.94	4.60±0.91	-	2.4±1.36	-	43.04±2.90	4.55(2)	0.29
	D2	44.0±1.33	1.16±1.00	-	14.4±1.66	15.96±4.84	43.18±4.30	2.98(1)	0.19
SS	N1	223.0±4.84	20.38±2.25	95.7±8.5	56.6±5.25	-	-	4.48(2)	0.27
	D1	221.1±5.49	20.30±2.06	47.9±10.5	15.1±5.77	-	-	0.47(2)	0.26
	N2	216.1±8.44	16.49±3.30	161.4±13.6	100.5±9.25	-	-	2.97(2)	0.28
	D2	170.8±3.23	13.31±1.89	108.8±5.8	87.8±3.62	33.91±7.24	-	1.66(1)	0.30
SF	N1	107.2±1.89	10.35±1.18	44.1±3.48	30.4±2.31	-	-	2.78(2)	0.32
	D1	179.3±3.14	6.67±1.20	120.5±5.87	60.4±3.05	-	-	2.47(2)	0.32
	N2	75.9±0.86	14.07±1.97	-	6.5±2.21	11.94±6.86	-	3.96(2)	0.33
	D2	103.3±1.75	2.95±1.49	15.5±3.65	-	-	-	4.27(3)	0.32
POD	N1	11.6±0.38	-	0.24±0.33	4.12±0.39	2.24±0.33	-	3.50(2)	0.21
	D1	12.5±0.53	3.45±0.18	7.6±0.92	2.89±0.56	-	-	4.11(2)	0.21
	D2	15.9±0.15	1.17±0.29	-	-	4.81±1.03	0.42±0.50	3.80(2)	0.26
CAT	N1	49.5±0.96	14.45±0.96	11.5±3.34	-	61.89±2.63	7.68±3.28	2.51(1)	0.20
	D1	74.5±0.25	17.24±0.87	-	18.4±0.89	54.52±3.39	-	3.11(2)	0.17
	N2	50.8±1.54	3.52±0.78	4.10±2.74	2.82±1.72	-	-	3.01(2)	0.26
	D2	72.3±0.39	8.11±0.97	-	7.16±1.05	19.13±3.50	-	1.69(2)	0.24
H_2O_2	N1	0.26±0.004	0.01±0.005	0.017±0.009	-	0.156±0.017	-	3.45(2)	0.27
	D1	0.41±0.007	0.02±0.005	0.108±0.012	0.09±0.008	0.177±0.016	-	2.80(1)	0.26
	N2	0.28±0.003	0.01±0.005	-	-	0.087±0.017	0.023±0.011	2.71(2)	0.27
	D2	0.36±0.001	0.04±0.004	-	0.04±0.004	0.11±0.014	-	3.66(2)	0.24
PRO	N1	0.54±0.006	0.04±0.008	-	0.104±0.01	0.061±0.033	0.004±0.016	2.24(1)	0.28
	D1	0.64±0.015	0.06±0.007	0.56±0.03	0.29±0.017	-	-	3.96(2)	0.31
	N2	0.65±0.002	0.06±0.009	-	0.067±0.009	0.091±0.024	-	2.82(2)	0.29
	D2	0.76±0.020	0.10±0.012	0.18±0.03	0.44±0.02	-	-	3.39(2)	0.36

Where, (m); mean, (d); additive effects, (h); dominance effects, (i); additive × additive effects, (j); additive × dominance effects, (l); dominance × dominance effects, S.E; standard error, χ^2; chi square, d.f; degree of freedom, h^2_{ns}; narrow-sense heritability, N1; cross I under normal condition, D1; cross I under drought condition, N2; cross II under normal condition, D2; cross II under drought condition, PH; plant height (cm), BP; no. of bolls per plant, SCY; seed cotton yield (g), SS; stomatal size (µm), SF; stomatal frequency, POD; peroxidase activity (U/mg), CAT; catalase activity (U/mg), H_2O_2; hydrogen peroxide (µmole/g) and PRO; proline content (µmole/g).

The dominance main effect (h) was quite higher than additive main effect (d) in Cross N1 but it was reduced in Cross D1. Non-allelic interaction *i.e.*, (i) and (j) type of interactions signified in Cross N1 which was missing in Cross D1. However, the presence of (l) type of epistatic interaction in Cross D1 was also observed. The models with four parameters (m, d, j, l) in Cross N2 and five parameters (m, d, h, i, j) in Cross D2 were good fit to data. Only one main effect *i.e.*, additive (d) type of gene action prevailed in Cross N2 along with non-allelic type of interaction like additive × dominance (j) and dominance × dominance (l). A change in gene action was prominently observed from normal to drought environment where dominance genetic effect (h) was observed in Cross D2. Additive × additive (i) type of interaction was also observed with additive × dominance (j) type of interaction. Therefore, the overall genetic control was reflected to dominance and variable epistatic interactions across two crosses and both water treatments which has increased the complexity of the trait. Narrow-sense heritability was estimated *i.e.*, 0.25, 0.22, 0.26 and 0.20 in Cross N1, D1, N2 and D2, respectively.

Seed cotton yield (SCY): The model good fitted to data of seed cotton yield in Cross N1 and D1 was four parameter model (m, d, h, j). The dominance effect (h) accounted with considerable magnitude as compared to additive (d) type of gene action under both water conditions. The non-allelic interaction *i.e.*, additive × dominance (j) also showed higher magnitude in Cross N1 and D1. The models with four parameters (m, d, i, l) in Cross N2 and five parameters (m, d, i, j, l) in Cross D2 were good fit to data of the trait. Dominance main effect (h) was missing under both water treatments (N2 and D2) but dominance × dominance (l) type of interaction with considerably larger magnitude than other effects was observed in them for SCY. (d) and (i) effects showed very small magnitude. The presence of epistatic effect *i.e.*, dominance × dominance (l) type of interaction was also signified in Cross N2 and D2. The trait was overall under influence of dominance effects in both cross combinations. Heritability estimation was observed to be 0.28 and 0.29 under normal condition in Cross N1 and N2 whereas it was 0.20 and 0.19 in Cross D1 and D2, respectively.

Stomatal size (SS): In case of stomatal size, the model with four parameters (m, d, h, i) in Cross N1, D1 and N2 was good fit to data. Additive main effect (d) and dominance main effect (h) were observed in these cross combinations. The presence of additive × additive (i) type of interaction was also signified. The magnitude of dominance effect (h) was larger than other effects in the model which explained the greater influence of non-additive effects controlling the genetic variability of the trait. In Cross D2, the model with five parameters (m, d, h, i, j) was good fit to data. The presence of additive × dominance (j) type of interaction was an addition to increase the chance of non-additive type of genetic variability in it. Therefore, the overall genetic control was under influence of dominance effects in the trait. Heritability estimation was observed to be 0.27 and 0.28 under normal conditions (N1 and N2) whereas it was 0.26 and 0.30 under drought conditions (D1 and D2), respectively.

Stomatal frequency (SF): The model *i.e.*, (m, d, h, i) was good fit to stomatal frequency in Cross N1 and D1. The genetic effects (d), (h) and (i) type of interaction signified in Cross 1 under both water treatments. Dominance effect (h) was quite higher than additive effect (d) and additive × additive effect (i). Hence, the trait was considered to be mostly controlled by non-additive effects. In Cross 2, the models with four parameters (m, d, i, j) under normal conditions (N) and three parameters (m, d, h) under drought conditions (D) were good fit to SF. Additive effect (d) was signified in Cross N2 because of the larger magnitude and absence of dominance effect (h). However, the presence of additive × dominance effect (j) in considerable magnitude reflected the occurrence of non-additive genetic variability. In Cross D2, only additive-dominance parameter model was good fit to data and no epistatic influence on the trait was observed. Dominance effect (h) was observed to be controlling the trait because of the larger magnitude of the effect. The overall impact of genetic effects directed the influence of non-additive effects for trait in both cross combinations. Heritability estimation was observed to be 0.32 and 0.33 in Cross N1 and N2 whereas it was 0.32 in Cross D1 and D2, respectively.

Peroxidase activity (POD): In this case, the good fitted models in Cross 1 were (m, h, i, j) under normal conditions (N) and (m, d, h, i) under drought conditions (D). Additive effect (d) was missing in Cross N1 but it was present in Cross D1. Dominance effect (h) and additive × additive (i) type of epistatic effect has been observed under both water treatments. The presence of additive × dominance effect (j) with dominance main effect (h) showed the occurrence of considerable non-additive genetic variability but additive × additive (i) type of non-allelic interaction showed larger magnitude. Therefore, the trait was governed by both additive effects (fixable genes) and dominance effects (non-fixable genes) in Cross N1. The magnitude of dominance main effect (h) was larger than additive effect (d) and additive × additive (i) type of epistatic effect in Cross D1. Concluding, the genetic control was under influence of non-additive effects. In Cross 2, generation mean analysis was not conducted under normal conditions (N) as generations showed no variability among them for peroxidase activity but four parameter model (m, d, j, l) was good fit in Cross D2. Additive main effect (d) and two epistatic effects *i.e.*, additive × dominance (j) and dominance × dominance (l) were observed in Cross D2. (j) type of interaction showed higher magnitude than (d) effect and (l) type of interaction. Moreover, dominance × dominance (l) effect also played a part on overall impact on genetic control of the trait. Therefore, the cumulative influence on genetic variability of the trait in both crosses was due to non-additive effects but a considerable response of additive effects was also observed. Heritability estimation was observed to be 0.21 in Cross N1 and D1 whereas it was 0.26 in Cross D2.

Catalase activity (CAT): Five parameter model (m, d, h, j, l) in Cross N1 and four parameter model (m, d, i, j) in Cross D1 were good fit to data of catalase activity. There was good magnitude of additive effect (d) in Cross N1 but the cumulative magnitude of dominance main effect (h) and two epistatic effects namely additive × dominance (j) and dominance × dominance (l) was higher than additive effects. So, non-additive effects prevailed in Cross N1. Dominance gene action (h) was missing in Cross D1. However, the presence of large magnitude of additive × dominance (j) type of effects prevailed the occurrence of non-additive genetic variability with considerable magnitude. The trait was observed to be governed under influence of dominance effects with considerable additive effects under drought condition. The models *i.e.*, (m, d, h, i) in Cross N2 and (m, d, i, j) in Cross D2 were good fit to data. Additive effect (d) and additive × additive (i) type of interaction has been observed under both water treatments in Cross 1 and 2. Dominance effect (h) was also observed in Cross N2 with higher magnitude than other effects in the model. In Cross D2, dominance effect (h) was missing but additive × dominance (j) type of epistatic interaction was present with larger magnitude. It reflected the influence of non-additive effects under drought condition. Therefore, the inheritance pattern of the trait in both crosses was under influence of non-additive effects with significant additive effects. Heritability estimation was observed to be 0.20 and 0.26 under normal conditions (N1 and N2) whereas it was 0.17 and 0.24 under drought conditions (D1 and D2).

Hydrogen peroxide content (H_2O_2): The data of hydrogen peroxide content was good fitted with the models (m, d, h, j) in Cross N1 and (m, d, h, i, j) in Cross D1. The presence of additive × dominance (j) type of genetic interaction with larger magnitude along with dominance main effect reflected towards the influence of non-additive effects prevailing on genetic variability of the trait in both water treatments (N1 and D1). The presence of additive effect (d) under both water regimes explained the occurrence of considerable additive effects (fixable genes). In Cross 2, the models (m, d, j, l) in normal conditions (N) and (m, d, i, j) in drought conditions (D) were good fit to data. Higher magnitude of epistatic interactions like additive × dominance (j) and dominance × dominance (l) than additive main effect (d) explained the influence of non-additive effects in Cross N2. The cumulative

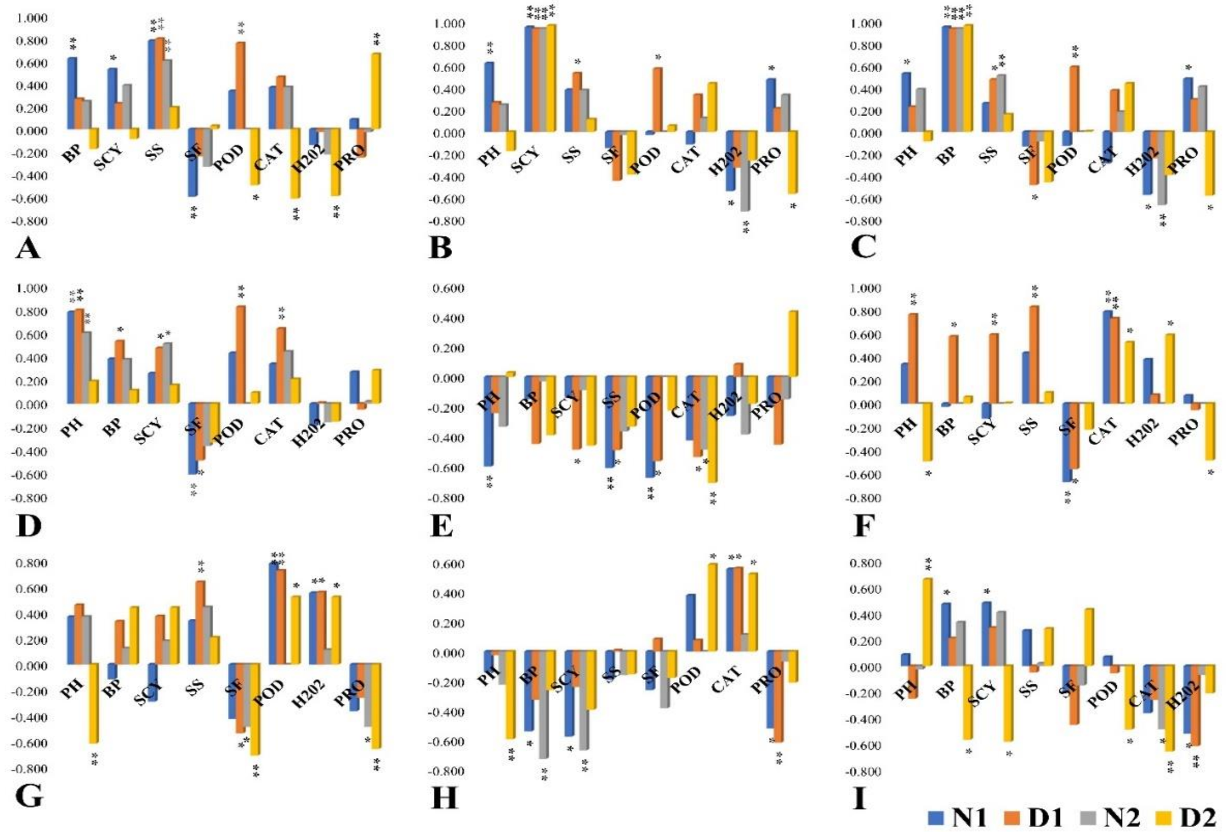

Figure 4. Interrelationship of (A.) plant height (PH), (B.) no. of bolls per plant (BP), (C.) seed cotton yield (SCY), (D.) stomatal size (SS), (E.) stomatal frequency (SF), (F.) peroxidase activity (POD), (G.) catalase activity (CAT), (H.) hydrogen peroxidase content (H_2O_2), (I.) proline content (PRO) with different traits in generations of Cross 1 (N1=Normal and D1=Drought) and Cross 2 (N2=Normal and D2=Drought). Columns in each subfigure show association of particular trait with different traits on positive (0 to 1) or negative (0 to -1) y-axis. Significance of association among traits is represented by labeling columns with * = Significant ($p<0.05$) and ** = Highly significant ($p<0.01$).

effect of additive (d) gene action and additive × additive (i) type of interaction was smaller in magnitude as compared to additive × dominance (j) type of interaction in Cross 2 under drought condition (D). It showed that the presence of non-additive effects concluded greater influence on genetic variability of the trait. Heritability estimation was observed to be 0.27 in both cross combinations under normal condition (N1 and N2) whereas it was 0.26 and 0.24 in Cross D1 and D2, respectively.

Proline content (PRO): The models with five parameters (m, d, i, j, l) in Cross N1 and four parameters (m, d, h, i) in Cross D1 were good fit to data. The presence of additive effect (d) and additive × additive (i) type of genetic interaction showed the presence of additive effects in Cross N1. However, presence of additive × dominance (j) and dominance × dominance (l) type of interactions showed the occurrence of non-additive variability. In Cross D1, dominance effect (h) was larger than other genetic effects. This observation explained the influence of non-additive effects on genetic control of the trait. In Cross 2, four parameter model (m, d, i, j) under normal conditions (N) and four parameter model (m, d, h, i) under drought conditions (D) were good fit to data. The presence of additive effect (d) and additive × additive (i) type of interaction reflected towards the occurrence of additive variability (fixable genes). On the other hand, presence of additive × dominance (j) type of interaction reflected the occurrence of non-additive effects (non-fixable genes) in Cross N2. Under drought conditions (D), the effect of dominance (h) was larger than additive effect (d) in Cross 2. This resulted into the influence of dominance effects on genetic control but the presence of additive × additive (i) type interaction reflected the occurrence of sum of fixable genes in large magnitude. So, the variability of the trait was controlled by both additive and non-additive effects. Heritability estimation was observed to be 0.28 and 0.29 in Cross N1 and N2 whereas it was 0.31 and 0.36 in Cross D1 and D2, respectively.

Interrelationship among drought related traits: Correlation coefficient analysis for various parameters is given in Fig. 4 for Cross combinations. PH showed positive association with BP in Cross N1. SCY showed highly positive association with BP in both cross combinations under both water treatments (Normal and drought). PH in Cross N1 were positively associated with SCY. SS was positively linked to SCY in Cross D1 and N2. The following traits also showed positive association with SS which are BP, POD and CAT in Cross 1. Negative correlation was only observed with SF in Cross 1 under both water regimes. POD was negatively correlated to SF in Cross N1 and D1. In Cross I, Negative association was also observed with PH Cross N1 and SCY in Cross D1. Moreover, CAT also showed negative correlation with SF in Cross D1, N2 and D2.

POD showed positive association with CAT in both cross combinations under both water treatments. POD activity was also positively correlated to PH, BP, SCY and SS in Cross D1 and H_2O_2 in Cross D2. PRO was negatively linked to POD in Cross 2 under water deficit environment. CAT was positively correlated to H_2O_2 in Cross 2 under drought conditions. PRO was also observed to be negatively linked to CAT in Cross N2 and D2. Negative association of H_2O_2 was observed with yield related traits like BP and SCY in both cross combinations under normal conditions. Proline content (under both water treatments) were observed to be negatively correlated to H_2O_2 in Cross 1. In Cross 2, PH also showed negative correlation with H_2O_2 under drought conditions. Correlation coefficient analysis showed positive association of PRO with BP and SCY in Cross N1. PH (in Cross 2) also showed positive association with PRO under drought conditions.

DISCUSSION

The presence of genetic variability in a breeding program is essential for the selection of potential plants with desirable attributes (Smith *et al*., 2015) which is related to the different allelic combinations among populations and within segregating populations (Ngangkham *et al*., 2018). Therefore, the availability of genetic variability and information related to gene actions controlling the traits are important for a successful breeding approach (Munir *et al*., 2007). In current research, the parameters under study showed significant genetic variability among different generations in both cross combinations under both water treatments which were also found by Malik *et al*. (2006), Sarwar *et al*. (2012), Saleem *et al*. (2015), Madhukar *et al*. (2018) and Zahid *et al*. (2021).

Among generations of cross combinations, F_1 hybrids showed highest mean values for yield contributing traits (PH, BP and SCY) indicating for the hybrid production of cotton (Iqbal and Nadeem, 2003). This idea was rejected because of the minimum reduction in mean values of the segregating populations due to drought stress. Thus, it has forced to assume that the cotton plant is equipped with complicated genetic architecture because of the presence of drought tolerance mechanism in segregating populations transferred from superior parents. Similar response of SS was observed as of yield related traits whereas SF behaved differently observing lowest performance of backcrosses and lowest % decline in F_1 and P_2 generations. Among biochemical attributes, drought sensitive parents (P_2) showed lower PRO while higher other biochemical traits. On contrary, drought tolerant parents (P_1) exhibited higher accumulation of PRO. The highest mean value was shown by BC_1 population but segregating populations showed higher % increase due to moisture stress. Similar responses for biochemical attributes against drought stress were also studied by Hasan *et al*. (2018) and Jie *et al*. (2020). Like PRO, most of the yield related traits, SS and H_2O_2 were also observed with lowest % reduction due to drought stress in backcrosses and 2nd filial generations which indicated the potential of selection for these traits from segregating populations. Majeed (2021) also reported similar behavior of segregating populations under heat stress tolerance in upland cotton on the base of pollen viability. Thus, the variability in aforementioned traits among different populations reflects to be inherited in complicated manner to exhibit drought tolerance. It is important to understand the genetic makeup of the traits for their improvement to cope with drought conditions more resistively directing towards an appropriate approach for estimation of genetic effects of traits related to yield and drought tolerance (Zdravkovic *et al*., 2011).

In generation mean analysis, the present study revealed that the genetic variability of the traits was controlled by variable genetic effects among generations of two cross combinations under moisture stress. The presence of higher magnitude of dominance main effect and epistatic effects like additive × additive, additive × dominance and dominance × dominance in morpho-physiological attributes like PH, BP, SCY, SS and SF explained the restriction of selection for these traits in early segregating populations. Iqbal and Nadeem (2003), Hussain *et al*. (2009), Sarwar *et al*. (2012) and Giri *et al*. (2020) also reported complex inheritance as appearance of non-additive effects with epistatic effects controlling genetic variability of yield related traits. Similar inheritance pattern exhibited by PH was also found by Saleem *et al*. (2015) while studying genetic effects of morpho-physiological attributes of upland cotton under water deficit environment. In physiological attributes (SS and SF), Amjid (2014) found relevant behavior and suggested for similar conclusions as of yield related traits. Correlation analysis revealed positive association among yield related traits which was in line with the findings of Rehman *et al*. (2020). This association reflected that the selection for one yield related trait would direct to the selection of another trait. This explains the genetic linkage among traits under study. On contrary, physiological traits *viz*., SS and SF associated reciprocally which means the increase in mean value of one trait would decrease the mean value of the other one. The association of SS and SF with yield related attributes was positively and negatively linked, respectively. Hence, this association would help a breeder to select high yield performing plants on the basis of the association with physiological traits and these findings were in accordance with Saleem *et al*. (2015).

At cellular level, different metabolites are affected by hydrogen peroxide content (included in ROS group) under drought stress. (Mittler, 2002). The accumulation of this content damages functional homeostasis of the cell (Fernandez-Ocana *et al*., 2011). Therefore, this content is not desirable in drought tolerant plants. The genetic effects exhibited by hydrogen peroxide were in accordance with a study conducted on maize crop under moisture stress by Sofalian (2017). In response to ROS production, the oxidative stress at cellular level is controlled with the help of antioxidant enzymes including POD and CAT (Ali and Ashraf, 2011). Therefore, plants having more accumulations of these antioxidants would perform better under drought stress environment. Non-additive variance was noted in larger magnitude and these results were similar to the findings of Abid *et al*. (2016). Proline content was controlled by both additive and non-additive genetic variance along with non-allelic interactions. Sofalian (2017) experienced similar response of proline content except for epistatic effects. Like morpho-physiological attributes, biochemical parameters have also reflected for selection of plants in later segregating populations for their better improvement. Additionally, low-moderate narrow-sense heritability is a sign of non-additive effects in large magnitude forcing towards the suggestions observed using genetic effects of the traits under study. The association among biochemical attributes was positively correlated. Particularly, the accumulation of hydrogen peroxide due to drought stress environment would increase the activity of antioxidants and proline content to prevent the oxidative damage to the cells which is desirable in drought tolerant plants. Negative association of hydrogen peroxide and positive association of antioxidants and, proline content with yield related traits reflected that the better performing plants under drought stress would have less cellular damage, vice versa. Similar association of biochemical attributes with yield related traits was also observed in a research conducted by Shavkiev *et al*. (2020). On the other hand, the presence of antioxidant enzymes and proline content in higher percentage directs to the drought tolerance of the plant. Feng *et al*. (2011), Ahmed *et al*. (2013) and Hasan *et al*. (2018) also studied the importance of biochemical attributes in drought tolerance and suggested these attributes as indicators for drought tolerance.

Conclusion: Mean performance of the traits reflected complex mechanism of inheritance in generations which led the research to conduct genetic analysis for drought tolerance in selected cotton genotypes. Thus, genetic effects of morpho-physiological traits (PH, BP, SCY, SS, SF) and biochemical attributes (POD, CAT, H_2O_2, PRO) were estimated revealing additive effect, dominance effect in large magnitude and non-allelic interactions along with low-moderate narrow-sense heritability. Hence, selection would be appropriate in later segregating generations for the improvement in current plant material for drought stress tolerance. Correlation studies revealed that the association of physiological and biochemical attributes with yield related traits could be exploited at the time of selection in later segregating generations. This study would be helpful for the development of drought tolerant varieties using further breeding programs.

Conflict of Interest: The Authors declare that there is no conflict of interest.

Authors' Contribution Statements: Musab Imtiaz conducted the research and wrote the 1st original draft. Amir Shakeel, as a supervisor edited the manuscript and gave final approval for publication. Azeem Iqbal Khan and Muhammad Shah Nawaz-ul-Rehman assisted in designing and statistical analyzation of the experiment.

Acknowledgment: Authors are thankful to the department of Plant Breeding and Genetics for providing the space in green house and field area for experiments conducted. Musab Imtiaz is also grateful to Higher Education Commission, Pakistan to provide the funding through Ph.D. scholarship Pin # 518-120647-2AV5-064.

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