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Generation Mean Analysis and Molecular Markers for Drought Tolerance in Wheat during Germination and Seedling Stage

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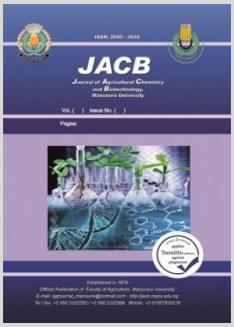

ABSTRACT

Generation mean analysis for drought tolerance was studied in wheat cross-1 (Pavon-76 X Gemmeiza-7) and cross-2 (ICR-DH18 X Pavon-76). The genotypes were evaluated under control and drought stress (15% polyethylene glycol 6000) at germination and seedling stage for seven traits. The additive–dominance model is adequate for explaining the inheritance of root and shoot lengths under both treatments and root fresh weight under drought stress in cross-1, and shoot length in both treatments and root length under drought stress in cross-2, while being inadequate in the other traits in the two crosses treatments. The additive-dominance and epistatic interaction effects recorded for germination percentage, root and shoot fresh and dry weights in both crosses suggested postponement of plant selections till the later generations for plant traits with such type of gene action. Epistasis absence and the contribution of considerable additive genetic variance in root and shoot lengths and root fresh weight indicating that recurrent selection in early segregating generations could be effective to select wheat lines with enhanced early tolerance to drought stress. Only two SSR primers and two TRAP primer pairs generated polymorphic bands from the tested genotypes. Four positive molecular markers were detected for drought tolerance. The UPGMA clustering analysis revealed correlation between drought tolerance genotypes and the studied molecular markers. The markers identified herein would allow implementing marker-assisted selection to screen wheat segregating populations for drought tolerance.

Keywords: *Triticum aestivum L., drought tolerance, generation mean analysis, bulk segregant analysis, PCR, SSR, TRAP.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the important crops in the world, contains starch, protein, sugar and provides food for human population (Peleg *et al.*, 2011; Liu *et al.*, 2016; Yu *et al.*, 2016). Drought affect wheat yield in arid ‘areas during crop season for short and long periods, so known as the most important abiotic stress which affects almost every aspect of plant growth through alterations in metabolism and gene expression (Leopold, 1990). Egypt is one of the countries that suffer severe drought and high temperature problems, especially in Upper Egypt. In addition, Egyptian wheat production is not sufficient to meet the demands of growing population. Enhancing drought tolerance is, hence, a major goal in plant breeding methods (Ehdaie *et al.*, 1991; Ehdaie and Waines, 1993).

Wheat often experiences drought stress at various growth stages especially during germination, tillering and early grain filling with corresponding depressions in biomass production and grain yield under drought conditions. The selection of drought tolerance genotypes are considered among the crucial in dry land areas as it cannot be controlled or easily apply the inducement drought stress in the field, in addition, there is no precise method for evaluating various genotypes under uncontrollable field conditions (Shaheen and Hood-Nowotny, 2005). Evidently, seedling growth parameters such as coleoptile, shoot and root length could be used as selection criteria for drought tolerance in wheat (Dhanda *et al.*, 2004; Rauf *et al.*, 2007; Bayoumi *et al.*, 2008; Datta *et al.*, 2011; Khakwani *et al.*, 2011; Baloch *et al.*, 2012). Moreover, the ratio between the average values of seedling traits in the control and stress conditions was used for evaluating drought tolerance (Srividya *et al.*, 2011). Polyethylene glycol (PEG) can be used as a drought simulator because of osmotic stress which causes it. (Turhan, 1997; Ashraf *et al.*, 2006).

it is important to identify the genetic structure of a set of parents and the pattern of gene action that controls traits related to drought tolerance causes breeding programs for drought tolerance to be more effective and successful (Badieh *et al.*, 2012). Several developments were carried out on the genetic models by many researchers for assessing different genetic effects (Gamil and Saheal, 1986; Kearsey and Pooni, 2004). However, most of these models based on simply additive – dominance effects. The analysis of generation means explained the contribution of additive-dominance effects and epistatic gene actions due to non-allelic gene interactions in the genotypic values of individuals, families and generations (Mather and Jinks, 1982). Generation mean analysis is valuable method for determining the genetic components and gene effects for the polygenic traits. Its greatest advantage lies in the ability to determine the epistatic gene effects such as “dominance x dominance”, “additive x additive” and “additive x dominance” effects.

The advent of PCR-based molecular markers for use as probes for genomic DNA has revolutionized the genetic analysis of crop plants and provided not only geneticists, but also agronomists and breeders with valuable new tools to

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identify traits of importance in improving resistance to drought stress (Quarrie, 1996; Quarrie *et al.,* 2003; Hu and Vick 2003; Raveena *et al.*, 2019). Among these markers, simple sequence repeats (SSRs) and target region amplified polymorphism (TRAP) provide excellent targets and means of assessing genetic variation among genotypes at the DNA level.

SSR are short nucleotide sequences (1-6 nucleotides) tandemly repeated from 2 – 10 times in the genome, such as (GT)n or (CT)n. While, TRAP markers based on fixed primer complementary to known DNA sequence and pairs it with arbitrary primer. The arbitrary primers having AT-or GC-rich core target to the exon or intron regions to amplify DNA fragments (Hu and Vick, 2003; Li *et al.,* 2006). The Bulk Segregant Analysis method (BSA) was developed to search for linked markers to the gene of interest, by comparing the PCR products of pooled DNA from several plants of a segregating population, i.e., high and low (Michelmore *et al.,* 1991). BSA was used by Altinkut and Gozukirmizi (2003), Quarrie *et al.* (2003) and Naroui Rad *et al.* (2012) to identify molecular markers linked to drought tolerance in wheat. The determination of positive markers for drought tolerance will facilitate the breeding programs for such character.

Thus, the present investigation aimed to analyze the genetic control of drought tolerance in bread wheat during germination and seedling stage using generation mean analysis in order to identify the most effective criteria, and proper breeding strategy for improving drought stress tolerance in wheat. In addition, bulk segregant analysis based on SSR and TRAP markers was carried out to identify molecular markers linked to drought tolerance in wheat.

MATERIALS AND METHODS

The experimental material consisted of the six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) derived from two wheat crosses. The First cross was between the local cultivar Gemmeiza-7 and Pavon-76 while the second cross was between ICR-DH18 and Pavon-76. Both Pavon-76 and ICR-DH18 are characterized as drought tolerant while Gemmeiza-7 was sensitive to drought stress (Table 1). The study was carried out at Genetics department and the experimental farm of Faculty of Agriculture, Assiut University, Egypt, during the period from 2015-2019.

Table 1. Pedigree and origin of the genotypes used in two bread wheat crosses.

Cross	Parental name	Pedigree	Drought	Origin
Cross-1	Gemmeiza-7	CMH74.630/5X//SERI82/3/AGENT	susceptible	Egypt
	Pavon-76	VCM//CNO/7C/3/KAL/BB	tolerant	Bangladesh
Cross-2	ICR-DH18	ICARDA	tolerant	ICARDA
	Pavon-76	CMH74.630/5X//SERI82/3/AGENT	tolerant	Bangladesh

Generation mean analysis:

In 2015/2016 season, the two crosses were made among the parents to produce F_1 hybrid seeds. In 2016/2017 season, the F_1 plants were selfed to produce F_2 seeds and backcrossed to their parents to produce BC_1 and BC_2 seeds. In 2017/2018, the six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of the two crosses were used to study the inheritance and to determine the genetic components, which control drought tolerance at germination and seedling stage in a laboratory experiment. Wheat grains of each genotype were sterilized by immersion in in 20% (v/v) commercial bleach (which contained 5.5% NaOCl) for 5 min, then rinsed three times with distilled water. Then, the sterilized grains were germinated in aluminum trays filled with washed and sterilized sand. Drought stress was simulated by the addition of PEG-6000 at concentrations of 0.0 and 15.0% (w/v) to the soil. For the control treatment distilled water was used. The genotypes were laid out in a randomized complete block design (RCBD) with three replicates under dark conditions for the first three days. The percentage of seed germination was recorded after three days and the seedlings were harvested after two weeks of culture. Growth parameters at seedling stage, namely root length (RL, cm), shoot length (SL, cm), root fresh weight (RFW, mg), shoot fresh weight (SFW, mg), root dry weight (RDW, mg) and shoot dry weight (SDW, mg) were measured on 15 plants for each parent and F_1, 30 plants for each F_2, BC_1 and BC_2 populations in each replicate. Dry weight (mg) was measured after drying samples at 70°C for 72 h in an oven.

Data analysis:

Information collected for the studied traits were subjected for analysis of variance as portrayed by Steel *et al.* (1997) to find variations between all six generations of both cross combinations. Drought stress susceptibility index (DSI) was calculated as for each genotype using the following equation according to (Fischer and Maurer, 1978).

Stress Susceptibility Index (SSI) = [1-(Ysi-Ypi)]/SI

Where, Ysi, is the performance of the genotype under stress treatment; Ypi, the performance of the genotype in the control treatment; SI that is stress intensity,

where:

$$SI = 1 - \left[\frac{\bar{Y}_S}{\bar{Y}_p}\right]$$

The scaling tests (A, B, C and D) were carried out to identify involvement of epistasis as indicated by Mather and Jinks (1982). If epistasis was present, analysis for estimation of non-allelic interaction was done for estimation of six parametric models of inheritance viz., the mean of all generation [m], additive effects [a], dominance effects [h], additive x additive interaction [i], additive x dominance [j] and dominance x dominance [l] interactions as mentioned by Hayman (1958). The basic genetic model (M, D, and H) was used when there was no epistasis in any trait. The genetic components of variance were then used to compute narrow-sense (H^2_n) and broad -sense (H^2_b) heritability. The average degree of dominance was calculated as follows:

Average degree of dominance = $(H/D)^{0.5}$.

Molecular markers

The drought sensitive variety (Gemmiza-7, P_1), the tolerant one (pavon76, P_2) and their F_1 in addition to the most sensitive 5 plants of BC_1 and the most drought tolerant 5 plants of BC_2 were subjected for SSR and TRAP analyses to determine genetic markers associated with drought tolerance in wheat.

Isolation of wheat DNA

Total Genomic DNA of studied genotypes was extracted from the fresh leaves of 2-weeks-old seedlings using CTAB protocol for plants (Murray and Thompson, 1980) with some modifications.

PCR analysis

Four SSR primers and 3 TRAP primer combinations (Table 2), obtained from (metabion international AG) were used to amplify the template DNA. Amplification reactions were carried out in 25µL volumes, containing (11.7 µl dH2O, 3 µL of 10x buffer, 3.0 µL of dNTPs (2.5 mM), 4 µL of Mg Cl_2 (25m M), 1.0µL forward primer, 1.0µL reveres primer (2.5 µL), 0.3 µL of Taq polymerase (5U/µL) and 2.0 µL of genomic DNA (50 ng /µL). Amplification was carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K.) with initial denaturation at 94°C 5 min and the following amplification cycles: (a) for TRAP analysis: 10 cycles of 1 min denaturation at 94°C, 1 min annealing at 35°C and 2 min extension at 72°C, 35cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 2 min extension at 72°C, (b) for SSR analysis: 45 cycles comprising 94°C for 60 seconds, annealing of primer for 60 seconds at 58-60°C and the extension for 60 seconds at 72°C. The final extension was carried out for 10 minutes at 72°C.

Table 2. Primer sequences and codes used for molecular markers.

Primer codes			Sequence (5' to 3')	Tm (°C)
SSR	SSR-4	F	AAGAGGCAGAGATGGAGTTC	61
		R	TCCCTGACACAGACGAGAT	
	SSR-5	F	TGCAAAGCATCACGGAGA	61
		R	ATACACGGTGGAAGTTGGC	
	SSR-8	F	TTTCTTCCGCATCAAGAGATCC	55
		R	CCTCAGGCTATGGCACAGAAT	
	Xbarc121	F	ACTGATCAGCAATGTCAACTGAA	50
		R	CCGGTGTCTTTCCTAACGCTATG	
TRAP	TRAP-1	F	TGAGTCCAAACCGGAAT	50
		R	TCACCCGCACCTTCTTCC	
	TRAP-9	F	TGAGTCCAA ACCGGAGC	50
		R	TCACCCGCACCTTCTTCC	
	TRAP-14	F	GAGTCCAAACCGGAGC	50
		R	CCC TCCACCAATCACAAT	

Electrophoresis

The amplified products were separated by horizontal gel electrophoresis unit using 2.5% for agarose gel. Electrophoresis was carried out under constant voltage of around 80V for approximately 3-3.5 hours. The banding patterns were visualized under Transilluminator (Ultra-Violet Product, Upland, CA, USA,).

Data analyses

Molecular markers were scored visually using the software package MVSP (MultiVariate Statistical Package) and DNA bands were scored as present (1) or absent (0). Cluster analysis was performed as the dendrogram based on un-weighted pair group method with arithmetic means (UPGMA) method using the Multi-Variate Statistical Package (MVSP).

RESULTS AND DISCUSSION

Wheat often experiences drought stress at various growth stages especially during germination, tillering and early grain filling with corresponding depressions in biomass production and grain yield under drought conditions. Several researchers had studied the effects of drought on germination and seedling development in wheat (Dhanda *et al*., 2004; Rauf *et al.,* 2007; Bayoumi *et al.,* 2008; Datta *et al.,* 2011; Khakwani *et al.,* 2011; Baloch *et al*., 2012; Ahmad *et al.,* 2013; El-Rawy and Hassan, 2014). Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator (Turhan, 1997; Ashraf *et al.,* 2006). Therefore, germination percentage (GP), shoot length (SL), root length (RL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) in P_1, P_2, F_1, F_2, BC_1 and BC_2 populations of the wheat cross-1 (Gemmeiza-7 x Pavon-76) and cross-2 (ICR-DH18 X Pavon-76) were evaluated under control and drought stress (15% polyethylene glycol 6000) (Table 3).

The analyses of variance (Table 4) revealed significant differences between the studied generations in all studied traits of the two crosses as well as between control and drought treatment, indicating the existence of genetic variation and possibility of selection for drought tolerance. The "genotypes x drought treatments" interaction was significant in RL, SL, RDW and SDW of coross-1 and all traits in cross-2 except RDW and SFW, displaying that the genotypes performed differently from control to drought stress.

The results also revealed that drought stress significantly decreased the performance of all studied traits of all wheat genotypes in the two crosses, as compared with the control treatment. Many studies used different concentrations of PEG to determine the tolerant genotypes of wheat and found that the growth of shoot and root at seedling stage were affected differently under various levels of drought stress according to the genotype (Zhu 2001, Dranda *et al*., 2004; Munns, 2005; Huang *et al.,* 2006; Van den Berg and Zeng, 2006; Rauf *et al.,* 2007; Bayoumi *et al.,* 2008; Singh *et al.,* 2008; Raziuddin *et al.,* 2010; Baloch *et al.,* 2012; Ahmad *et al.,* 2013). PEG-induced stress significantly reduced shoot and root lengths due to interference in cell division and growth (Raziuddini *et al.,* 2010).

The lowest mean values of drought susceptibility index (DSI) were found for P_2 (Pavon-76, 0.86), F_1 (0.93), BC_1 (0.94) and F_2 (0.95) in cross-1 and for P_1 (ICR-DH18, 0.82), F_1 (0.90) and P_2 (Pavon-76, 0.95) in cross-2 (Table 3). This indicated that they possessed favorable genes for drought tolerance and selection in the segregation population for drought tolerance could be effective to produce wheat lines with high tolerance to early drought stress.

Table 3. The mean values of germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg), Shoot dry weight (SDW, mg) and Drought Susceptibility Index (DSI) in six basics for cross-1 (Gemmeiza-7 x Pavon-76) and Cross-2 (ICR-DH18 x Pavon-76) under control (C) and drought (D) stress (15 % polyethylene glycol 6000).

Populations	GP		RL		SL		RFW		RDW		SFW		SDW		DSI
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	
Cross-1 (Gemmeiza-7 x Pavon-76)															
P_1	92.50	72.50	16.48	6.79	20.87	6.11	90.00	58.40	15.95	6.50	92.40	66.60	29.35	17.10	1.16
P_2	97.50	87.50	11.20	5.42	16.93	5.82	68.00	50.40	12.00	7.25	61.00	45.70	11.95	8.05	0.87
F_1	95.00	82.50	13.25	6.39	17.50	5.91	70.50	51.25	12.20	6.95	60.00	44.50	13.1	8.70	0.93
F_2	92.00	75.00	12.65	6.32	17.08	5.87	69.75	50.95	11.90	5.90	58.70	44.00	12.95	8.35	0.95
Bc_1	94.00	78.00	11.18	5.93	17.82	6.13	65.45	52.00	13.00	6.45	60.00	45.65	10.5	6.70	0.94
Bc_2	86.00	72.00	15.43	7.16	20.48	6.68	84.75	56.20	14.25	5.30	84.85	60.45	21.45	10.95	1.09
L.S.D 0.05	5.07	10.02	0.84	0.71	0.46	0.22	9.36	8.89	3.79	2.85	8.56	7.13	4.39	3.66	0.87
Cross-2 (ICR-DH18 x Pavon-76)															
P_1	100.0	95.00	13.85	8.42	18.03	7.67	62.50	48.95	12.75	7.85	68.50	51.75	12.65	8.25	0.83
P_2	100.0	90.00	11.40	6.23	14.44	6.02	64.50	50.10	10.00	6.20	62.25	47.75	11.45	7.25	0.94
F_1	97.50	95.00	12.03	6.42	15.68	6.36	62.50	48.50	11.50	7.50	70.00	52.75	13.25	8.60	0.90
F_2	92.00	88.00	11.82	5.16	15.42	5.95	61.50	47.50	10.50	5.85	69.75	51.75	13.00	8.25	1.03
Bc_1	98.00	88.00	14.78	7.00	17.96	6.96	67.50	51.25	10.10	6.60	71.00	52.05	18.00	10.25	1.09
Bc_2	96.00	84.00	13.28	6.50	15.75	6.13	68.25	52.25	9.50	5.00	63.25	46.75	12.75	8.30	1.20
L.S.D 0.05	4.66	5.19	0.65	0.83	0.92	0.47	1.42	0.86	2.82	0.74	2.92	1.29	1.68	0.90	

Table 4. The analyses of variance for germination percentage (GP), shoot length (SL), root length (RL), Root fresh weight (RFW), Root dry weight (RDW), Shoot fresh weight (SFW) and Shoot dry weight (SDW) in six populations of cross-1 (Gemmeiza-7 x Pavon-76) and Cross-2 (ICR-DH18 X Pavon-76) under control (C) and drought (D) stress.

S.V.	DF	Mean of Squares						
		GP	RL	SL	RFW	RDW	SFW	SDW
Cross-3 (Gemmeiza-7 x Pavon-76)								
Replicates	2	46.02	0.02	0.21**	135.6^*	0.08	499.88**	48.00**
Genotypes	5	111.36**	2.83**	2.92**	250.91**	2.65**	803.21**	173.79**
Drought stress	1	2185.56**	692.82**	1536.39**	4438.89**	441.00**	3280.43**	424.36**
Geno. X Stress	5	20.46	0.60*	1.79**	56.20	5.44**	35.93	19.39**
Error	22	17.75	0.17	0.04	28.31	0.59	17.10	4.53
Cross-2 (ICR-DH18 x Pavon-76)								
Replicates	2	16.33	0.11	0.33	9.72**	1.96	6.53*	0.70
Genotypes	5	112.60**	5.94**	6.52**	31.16**	7.21**	58.00**	15.80**
Drought stress	1	625.00**	331.31**	871.24**	1944.81**	160.66**	2598.45**	228.01**
Geno. X Stress	5	31.15**	1.52**	1.35**	2.40**	0.44	3.42	2.71**
Error	22	6.70	0.15	0.16	0.39	1.25	1.66	0.50

Scaling test

As analysis of variance for the two crosses indicated dissimilarities for all genotypes for all studied traits, generation mean analysis was carried out for assessment of gene action for the seven traits in control and drought treatments. Analysis of generation means having scaling test is very important to find out either non-allelic gene action is present or not and which model for this analysis is suitable (Mather and Jinks, 1982; Sharmila *et al.*, 2007). The significance of any scaling test (A, B or C) indicated the presence of epistasis for the trait. Meanwhile, the non-significant scaling test reflects the absence of epistasis and the adequate model of three parameters is applicable. The 6 parameters can be applied in this situation to determine the heredity effects and epistatic gene action..

Table (5) showed that additive–dominance model is adequate for explaining the inheritance of RL and SL under both treatments and RFW under drought stress in cross-1.

Also, SL in both treatments and RL under drought stress in cross-2, indicating the simple genetic variation controlling the inheritance of these traits. Meanwhile, the model is inadequate in the other traits in both treatments of the two crosses, indicating the presence of non-allelic gene interaction for these traits.

Table 5. Scaling test for germination percentage (GP), shoot length (SL), root length (RL), Root fresh weight (RFW), Root dry weight (RDW), Shoot fresh weight (SFW) and Shoot dry weight (SDW) in six populations of cross-1 (Pavon-76 * Gemmeiza-7) and Cross-2 (ICR-DH18X Pavon-76) under control (C) and drought (D) stress.

Traits	Control			Drought stress		
	A	B	C	A	B	C
Cross-3 (Pavon-76 * Gm-7)						
GP	-4.50**	-15.50**	-12.00**	-14.00**	-11.00**	-25.00**
RL	-2.08	1.14	-3.58	1.10	1.16	1.27
SL	1.21	2.60	1.21	0.60	1.3ٕ	0.86
RFW	-7.60**	9.00**	-20.00**	2.35	2.75	-7.50
RDW	1.80**	0.35*	-4.75**	-1.30**	-2.85**	-4.05**
SFW	-1.50	16.80**	-39.60**	1.10	9.80**	-25.30**
SDW	-4.05**	0.45	-15.70**	-3.45**	-3.90**	-9.15**
Cross-2 (ICR-DH18 X Pavon-76)						
GP	-1.50	-5.50**	-27.00**	-18.00**	-23.00**	-39.00**
RL	3.69**	3.14*	-2.03	-0.90	0.38	-3.04
SL	2.21	1.65	-2.28	-0.05	-0.04	-2.35
RFW	10.00**	9.50**	-6.00**	5.05**	5.90**	-6.05**
RDW	-4.05**	-2.50**	-3.75*	-2.15**	-3.70**	-5.65**
SFW	3.50**	-5.75**	8.25**	-0.40	-7.00**	2.00**
SDW	10.10**	0.80*	1.40	3.65**	0.75**	0.30

In cross-1, A, B and C were important for GP and RDW in both treatments, RFW in the control, and SDW under drought stress. The B and C types were important in SFW under both treatments while the A and C types of

scaling test were important for SDW in the control treatment. In cross-2, A, B and C were important for RFW and RDW in both treatments, SFW in the control and GP under drought stress. The A and B types were important for RL in the control and for SDW in both treatments, while SFW showed the importance of B and C under drought stress. These results may be taken as an evidence for the failure of simple genetic model to ascertain the genetic variation for these traits in the corresponding crosses. Therefore, the six parameters model [m, d, h, I, j and l] was applied for these traits in order to assess the di-genic interaction types controlling the genetic variations. These results were in agreement with those of Sirvastava *et al.* (1992), Abd El-Mageed (2005), El-Sayed and El-Shaarawy (2006) and El-Aref *et al.* (2011).

Types of gene effects and components of variances

The mean parameter (m) for all studied attributes of the two crosses under control and drought stress was significant in all traits which reflect the contribution due to the overall mean plus the locus effects and interaction of the fixed loci (Tables 6 and 7). Additive gene effects [d] were significant for all traits under control and drought treatments in the two crosses, except SL under drought stress in cross-1, RL and RDW in the control treatment of cross-2. These results reflect the importance and effectiveness of additive effect in improving the performance of these traits using the pedigree selection program.

In cross-1, the estimates of dominance gene action [h] were significant in all traits under control treatment, except RL and SL, while under drought stress, only SFW and RFW displayed significant dominance effect [h]. In cross-2, dominance gene actions [h] were significant for all traits under control treatment, except RDW, and significant in all traits under drought stress, except SL and RL. These results indicated the importance of dominance gene effects in the inheritance of these traits.

Estimates of epistatic gene effects

Estimates of non-allelic gene effects were also determined and presented in Tables (6 and 7). In the control treatments, the three types of non-allelic gene interactions (I, j, l) were significant in GP, SFW, SDW, RFW and RDW of cross-1, and in GP, SFW and SDW of cross-2. Under drought stress, the three types of gene interactions are important for SFW of cross-1, and for GP, RFW, RDW and SDW of cross-2.

However, the additive x additive [i] and dominance x dominance [1] were significant in cross-2 for RL and RFW in the control, SFW under drought stress with predominant of [l] effects. RDW of cross-1 displayed significant additive x dominance [j] and dominance x dominance [1] interactions under drought stress with predominant of [l] effects. Only dominance x dominance [1] effects was significant for GP and SDW of cross-1 under drought stress, RDW of cross-2 in the control treatment.

The additive-dominance along with epistatic interaction effects recorded for GP, RFW, RDW, SFW and SDW in both crosses suggested postponement of plant selections till the later generations for plant traits with such type of gene action (Iqbal *et al.*, 2012; Khan *et al.*, 2016). Sharmila *et al.* (2007) and Said (2014) reported that the additive effects and gene interaction dominance x dominance (l) or other type di-genic complementary gene interaction can be exploited effectively by selection for the characters improvement.

The magnitude and significance of the estimates for [i], [j] and [l] indicated that epistatic genes are important in the basic mechanism of seedling traits involved in drought tolerance inheritance of the studied wheat crosses. Hayman (1960) has indicated when epistasis is of major importance in the inheritance of a trait, then it is impossible to obtain unbiased estimates of pooled additive or dominance effects. Also, the presence of both additive and non-additive effects in the genetic control of the traits in these crosses suggested that recurrent selection followed by pedigree breeding or a selective mating system may be useful in improving the tolerance of drought in wheat (Dehdari *et al.*, 2007).

Genetic variance of three parameters model

Root length of cross-1 displayed predominance of additive variance (D) over dominance variance (H) in both treatments indicating partial dominance [$(H/D)^{0.5}$ = 0.80, 0.48] (Table 6). The absence of epistatic effects in RL in conjunction with moderate (46.72%, control) to high narrow-sense heritability (67.21%, drought) suggested that recurrent selection in the segregation population for drought tolerance could be effective to produce wheat lines have improved RL and high tolerance to early drought stress. While, RL in cross-2 showed the importance of both additive and non-additive variances under drought stress with slightly over-dominance [$(H/D)^{0.5}$ = 1.06] and less values of heritability ($H^2_{b.s}$ = 44.73%, $H^2_{n.s}$ = 28.55%) suggesting that recurrent selection for RL may be valuable within cross-1 population than cross-2 (Table 7).

Additive gene effects for shoot and root dry weights, root length, and root/shoot ratio were reported by Ashadusjaman *et al.* (2012). In addition, predominance of additive effects have been reported for root length under stressed and non-stressed conditions, while the coleoptile length was governed by over dominance under stress conditions (Najafabadi *et al.*, 2004).

Shoot length in both crosses displayed predominance of dominance variance (H) over additive (D) in the control and *vice versa* under drought stress reflecting over- [$(H/D)^{0.5}$ =1.51] and partial-dominance [$(H/D)^{0.5}$ =0.53], respectively (Tables 6 and 7). While, the narrow sense heritability under stress (64.43%) was higher than that of the control treatment (34.32%). This suggested that selection for SL would be more valuable under drought than favorable conditions. El-Rawy and Hassan (2014) studied a diallel analysis of drought tolerance at seedling stage in wheat and found significant additive and dominance variances in RL, SL, root/shoot ratio and seedling dry weight of wheat genotypes under drought stress. They also found that over-dominance was involved in the inheritance of these traits. Low to moderately narrow-sense heritability was obtained for RL (0.18 and 0.12) and SL (0.19 and 0.12) at 15 and 20% PEG, respectively; root/ shoot ratio (0.15) and seedling dry weight (0.16) at 15% PEG (El-Rawy and Hassan, 2014).

In addition, simple genetic variation also control the inheritance of RFW under drought stress in cross-1which displayed predominance of additive variance [$(H/D)^{0.5}$ = 0.42] and higher value of narrow sense heritability (86.96%) suggesting that recurrent selection under drought stress will improve this trait.

Narrow-sense heritability estimates under drought stress in cross-1 (RL =67.21%, SL= 64.43%) and cross-2 (SL= 57.47%) were higher than those of cross-1 (RL

=46.72%, SL= 34.32%) and cross-2 (SL= 37.87%) in the control treatment. Increase in narrow sense heritability under drought stress than control is due to increase in additive variance that might be resulted due to expression of additional or hidden genes under drought stress that would not be expressed in non-stressed treatment (Shannon, 1984).

The absence of epistasis in addition to the contribution of considerable additive genetic variance in RL, SL and RFW indicating that recurrent selection in early segregating generations could be effective to select wheat lines with enhanced early tolerance to drought stress. When both additive and non-additive effects are involved in the control of the traits., reciprocal recurrent selection or Bi-parental mating can be used to improve these traits (Sharmila et al., 2007 and Said, 2014)

Many studies stated that seedling growth parameters including coleoptile, shoot length and root length can be used in the selection programs to improve drought tolerance in wheat (Hakizimana *et al.,* 2000; Dhanda *et al.,* 2004; Rebetzke *et al.,* 2007; Bayoumi *et al.,* 2008; Awan *et al.,* 2011; Datta *et al.,* 2011; Baloch *et al.,* 2012).

Table 6. Additive dominance analysis for germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg) and Shoot dry weight (SDW, mg) in six basics for cross-1 (Gemmeiza-7 x Pavon-76) on control (C) and drought stress.

Param and Variance components	Drought stress	GP	RL	SL	RFW	RDW	SFW	SDW
M	C	92.00**	14.94**	17.2٠**	69.75**	11.90**	58.70**	12.95**
	D	75.00**	7.33**	4.87*	41.8٠**	5.90**	44.00**	8.35**
[d]	C	8.00**	-0.84*	-1.07**	-19.30**	-1.25**	-24.85**	-10.95**
	D	6.00**	-0.69**	-0.14	-4.00**	1.15**	-14.80**	-4.30**
[h]	C	-8.00**	-0.38	3.83	12.90**	5.12**	38.70**	4.55**
	D	2.50	-2.1٠	4.04	27.15*	-0.03	24.55**	-2.08
[i]	C	-8.00**			21.40**	6.90**	54.90**	12.10**
	D	0.001				-0.10	36.20**	1.80
[j]	C	5.50**			-8.30**	0.73**	-9.15**	-2.25**
	D	-1.50				0.77*	-4.35**	0.23
[l]	C	28.00**			-22.80**	-9.05**	-70.20**	-8.50**
	D	25.00**				4.25**	-47.10**	5.55**
D	C		10.28	9.69				
	D		17.86	14.27	96.53			
H	C		6.60	22.03				
	D		4.15	4.07	17.07			
E	C		4.21	3.77				
	D		3.32	2.92	2.97			
h n	C		46.72	34.32				
	D		67.21	64.43	86.96			
h b	C		61.70	73.32				
	D		75.02	73.63	94.65			
Degree of dominance	C		0.80	1.51				
	D		0.48	0.53	0.42			

Table 7. Additive dominance analysis for germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg) and Shoot dry weight (SDW, mg) in six basics for cross-2 (ICR-DH18 x Pavon-76) on control (C) and drought stress.

Param and Variance components	Drought stress	GP	RL	SL	RFW	RDW	SFW	SDW
M	C	92.00**	11.82**	10.15**	61.5**	10.50**	69.75**	13.00**
	D	8.25**	4.82*	4.59**	47.50**	5.85**	51.75**	8.25**
[d]	C	2.00*	1.50	1.73**	-0.75*	0.60	7.75**	5.25**
	D	1.95**	1.09**	0.83**	-1.00**	1.60**	5.30*	1.95**
[h]	C	17.50**	8.27**	15.53*	24.50**	-2.68	-5.88**	10.70**
	D	4.95**	3.65	3.83	15.98**	0.28**	-6.40**	4.95*
[i]	C	20.00**	8.87**		25.50**	-2.80	-10.50**	9.50**
	D	4.10**			17.00**	-0.20**	-9.40**	4.10*
[j]	C	2.00*	0.28		0.25	-0.78	4.63**	4.65**
	D	1.45**			-0.43**	0.77**	3.30	1.45**
[l]	C	-13.00**	-15.70**		-45.00**	9.35**	12.75**	-20.40**
	D	-8.50**			-27.95**	6.05**	16.8**	-8.50**
D	C			10.34				
	D		6.65	8.08				
H	C			15.48				
	D		7.54	1.62				
E	C			4.61				
	D		6.43	2.58				
h n	C			37.87				
	D		28.55	57.47				
h b	C			66.21				
	D		44.73	63.24				
Degree of dominance	C			1.22				
	D		1.06	0.45				

Ahmed *et al.* (2019) reported that selection for RL, RFW, RDW, chlorophyll b and cell membrane thermo-stability at the seedling growth stage will improve genetic gain for wheat drought tolerance. They reported that the high performing genotypes under drought stress may be useful in future breeding programs, and early selection for

the recommended traits will be effective for developing drought-tolerant wheat varieties with high yield.

Estimated different types of gene effects provided a test for gene action and are useful for analyzing the genetic architecture of drought tolerance at seedling stage so as to further improve desirable traits. We may conclude that RL, RFW and SL can be vital for effective screening and selection of wheat genotypes at seedling-stage for drought-stress.

Molecular Markers

Drought stress tolerance in wheat is a quantitatively inherited trait controlled by several genetic loci, and several of its genetic components are difficult to measure (Forster *et al.,* 2000; Raveena *et al.,* 2019). Identification of associated molecular markers at a major locus contributing to drought stress tolerance would be useful for the indirect selection of wheat plants for drought tolerance (Visser, 1994; Raveena *et al.,* 2019). Molecular markers linked to a trait of interest can be identified by the use of bulk segregant analysis (BSA; Michelmore *et al.,* 1991), a technique that consists of pooling DNA of genotypes exhibiting extreme phenotypes (i.e., high and low) of a trait in a segregating population (such as BC_1, BC_2). If one selects for drought tolerance, the presence of polymorphism between the amplification patterns of the two bulks is expected only for those bands that are genetically linked to the genes controlling drought tolerance (Michelmore *et al.,* 1991; Naroui Rad *et al.,* 2012).

In the present study, the drought sensitive variety (Gemmiza-7, P_1), the tolerant one (pavon76, P_2) and their F_1 in addition to the most sensitive 5 plants of BC_1 and the most drought tolerant 5 plants of BC_2 were subjected for molecular marker analysis to determine genetic markers associated with drought tolerance in wheat. Four SSR primers and three TRAP primer combinations were used while only two SSR primers (SSR-5 and SSR-8) and two TRAP primer pairs (TRAP-9 and TRAP-14) generated polymorphic bands form the tested genotypes (Figs. 1and 2).

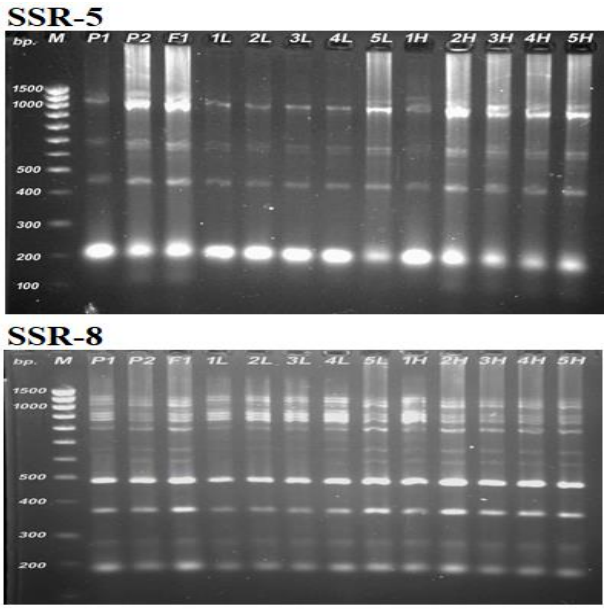

Fig. 1. Agarose gel electrophoresis of SSR markers generated by primers SSR-5 and SSR-8 in the two parents Gemmeiza-7 (P_1) and Pavon-76(P_2), their F1, 5 lowest drought sensitive plants from BC_1 (1L – 5L) and 5 highest drought tolerant plants (1H – 5H) from BC_2.

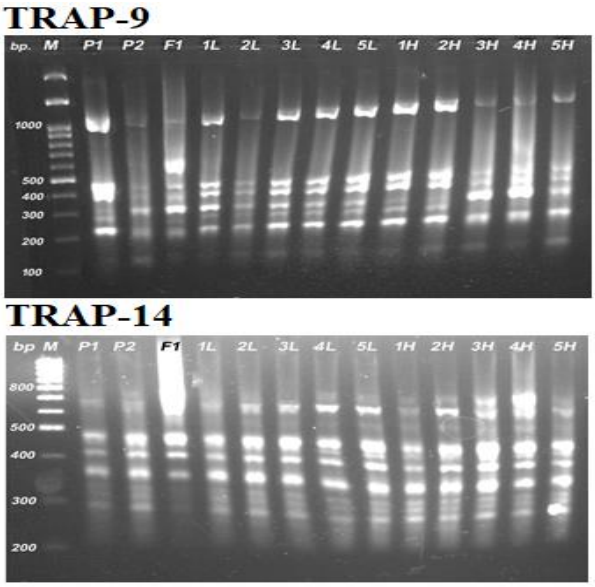

Fig. 2. Agarose gel electrophoresis of TRAP markers generated by primers TRAP-9 and TRAP-14 in the two parents Gemmeiza-7 (P_1) and Pavon-76 (P_2), their F1, 5 lowest drought sensitive plants from BC1 (1L – 5L) and 5 highest drought tolerant plants (1H – 5H) from BC_2.

These primers amplified a total of 37 DNA fragments ranged in size from 120bp (SSR-5) to 1300bp (SSR-8) (Tables 8 and 9). The least number of amplified DNA-bands was detected for the primer SSR-5 (6 bands), while the highest number was amplified by SSR-8 (14 bands), with an average of 9.25 bands/primer (Tables 8 and 9). Only 21.6% (8/37 bands) of these bands were polymorphic while 78.4% (29/37 bands) were common between the tested genotypes.

As to drought tolerance, four positive molecular markers were detected for that character. Two of them (120 and 1200 bp) were generated by SSR-5, one (600 bp) amplified by SSR-8 and the fourth (740 bp) generated by (TRAP-14) (Tables 8 and 9). These four markers are present in the tolerant parent (Pavon 76), F_1 and tolerant BC_2 genotypes, while mostly absent in the sensitive BC_1 genotypes and their parents.

The two DNA fragments 980bp (SSR-8), 260bp (TRAP-9), were observed in the drought sensitive parent (Gemmiza-7, P_1) and the sensitive plants of BC_1 in addition to one or two plants of BC_2. These results indicated that the polymorphism in these bands may be due to genetic segregation rather than association with drought tolerance. In addition, the DNA fragment 440bp (TRAP-14) not present in the two parents while detected in all BC_2 plants and 2 of BC_1 plants reflecting that it may be due to recombination rather than mutation.

The presence/absence data of the four primers was used for UPGMA clustering analyzed by MVSP software program according to Nie and Li (1979). The dendrogram tree showed that all genotypes were clustered together in two main clusters with a branched-off 0.847 genetic similarity (Fig. 3). The first main cluster included the drought sensitive parent (Gemmiza-7, P_1) and the sensitive plants of BC_1 (1L, 2L, 3L and 4L) with an average of 0.939 genetic similarity. The 2nd main cluster included the drought tolerance genotypes, P_2 (Pavon-76) and all tolerant BC_2 plants in addition to the F_1 within a branched-off 0.907 genetic similarity. These results

revealed close correlation between drought tolerance in these genotypes and the studied molecular markers, and the importance of these markers in breeding programs to select drought tolerant genotypes.

The markers identified in the present study [120 bp (SSR-5), 1200 bp (SSR-5), 600 bp (SSR-8) and 740 bp (TRAP-14)] would allow implementation of marker-assisted selection to screen wheat segregating populations for drought tolerance. However, to determine the transportability of this marker to other genotypes, other crosses derived from different parental genotypes may be evaluated. Similar conclusion was also reached by Altinkut and Gozukirmizi (2003), Quarrie *et al.* (2003) and Naroui Rad *et al.* (2012).

Table 8. Survey of SSR markers generated by primers SSR-5 and SSR-8 in the two parents Gemmeiza-7 (P_1) and Pavon-76(P_2), their F1, 5 lowest drought sensitive plants from BC_1 and 5 highest drought tolerant plants from BC_2.

No.	Molecular Markers	bp.	P_1	P_2	F_1	---------- BC_1 -------					---------- BC_2 --------				
						1L	2L	3L	4L	5L	1H	2H	3H	4H	5H
1	SSR-5	1200	0	1	1	0	0	0	0	1	1	1	1	1	1
2		1050	1	1	1	1	1	1	1	1	1	1	1	1	1
3		690	1	1	1	1	1	1	1	1	1	1	1	1	1
4		460	1	1	1	1	1	1	1	1	1	1	1	1	1
5		210	1	1	1	1	1	1	1	1	1	1	1	1	1
6		120	0	1	1	0	0	0	0	0	0	1	1	1	1
1	SSR-8	1300	1	1	1	1	1	1	1	1	1	1	1	1	1
2		1200	1	1	1	1	1	1	1	1	1	1	1	1	1
3		1100	1	1	1	1	1	1	1	1	1	1	1	1	1
4		980	1	0	1	1	1	1	1	1	0	1	0	0	0
5		920	1	1	1	1	1	1	1	1	1	1	1	1	1
6		900	1	1	1	1	1	1	1	1	1	1	1	1	1
7		870	1	1	1	1	1	1	1	1	1	1	1	1	1
8		800	1	1	1	1	1	1	1	1	1	1	1	1	1
9		650	1	1	1	1	1	1	1	1	1	1	1	1	1
10		600	0	1	1	0	0	0	0	1	1	1	1	1	1
11		490	1	1	1	1	1	1	1	1	1	1	1	1	1
12		370	1	1	1	1	1	1	1	1	1	1	1	1	1
13		280	1	1	1	1	1	1	1	1	1	1	1	1	1
14		190	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 9. Survey of TRAP markers generated by primers TRAP-9 and TRAP-14 in the two parents Gemmeiza-7 (P_1) and Pavon-76 (P_2), their F1, 5 lowest drought sensitive plants from BC_1 and 5 highest drought tolerant plants from BC_2.

No.	Molecular Markers	bp.	P_1	P_2	F_1	---------- BC_1 ----------					---------- BC_2 ---------				
						1L	2L	3L	4L	5L	1H	2H	3H	4H	5H
1	TRAP-9	950	1	1	1	1	1	1	1	1	1	1	1	1	1
2		580	0	0	1	0	0	0	0	0	0	0	0	0	0
3		450	1	1	1	1	1	1	1	1	1	1	1	1	1
4		390	1	1	1	1	1	1	1	1	1	1	1	1	1
5		310	1	1	1	1	1	1	1	1	1	1	1	1	1
6		260	1	0	1	1	1	1	1	1	1	1	0	0	0
7		225	1	1	1	1	1	1	1	1	1	1	1	1	1
8		130	1	1	1	1	1	1	1	1	1	1	1	1	1
1	TRAP-14	740	0	1	1	0	0	0	1	1	1	1	1	1	1
2		680	1	1	1	1	1	1	1	1	1	1	1	1	1
3		480	1	1	1	1	1	1	1	1	1	1	1	1	1
4		440	0	0	0	0	0	0	1	1	1	1	1	1	1
5		420	1	1	1	1	1	1	1	1	1	1	1	1	1
6		380	1	1	1	1	1	1	1	1	1	1	1	1	1
7		350	1	1	1	1	1	1	1	1	1	1	1	1	1
8		330	1	1	1	1	1	1	1	1	1	1	1	1	1
9		290	1	1	1	1	1	1	1	1	1	1	1	1	1

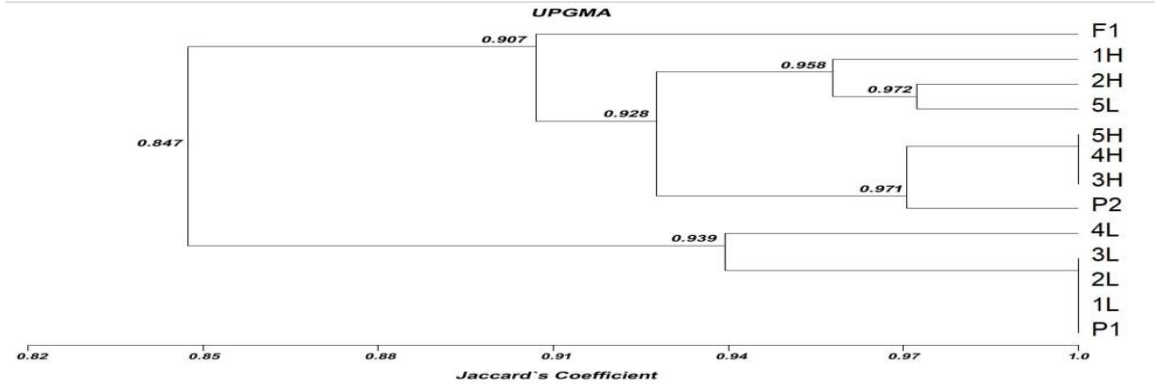

Fig. 3. Dendrogram demonstrating the relationship between the lowest drought sensitive plants of BC_1 (1L – 5L), highest drought tolerant plants (1H – 5H) of BC_2 as compared to their low (Gemmeiza-7, P_1) and high (Pavon-76, P_2) parents and their F_1

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تحليل متوسطات الأجيال والواسمات الجزيئية لتحمل الجفاف فى القمح خلال مرحلتى الإنبات والبادرات

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تمت دراسة تحليل متوسطات الأجيال الستة لتحمل الجفاف في القمح على الهجين الأول (Pavon-76 X Gemmeiza-7) والهجين الثانى (ICR-DH18 X Pavon-76). تم تقييم الطرز تحت ظروف الكنترول وظرف الإجهاد (١٥ ٪ البولي إيثيلين جليكول ٦٠٠٠) فى مرحلتى الإنبات والبادرات باستخدام سبعة صفات. وقد انطبق نموذج الإضافة- سيادة لصفتى طول الساق والجذر في الكنترول و إجهاد الجفاف ، ولصفة الوزن الرطب للجذر تحت إجهاد الجفاف بالنسبة للهجين الأول وانطبق النموذج بالنسبة للهجين الثانى على صفة طول الساق تحت كلا المستويين وطول الجذر تحت مستوى الإجهاد. بينما لم ينطبق النموذج على باقى الصفات تحت كلا المستويين فى كل من الهجينين. ووجود نموذج الإضافة- سيادة مع التفاعل الجينى لصفات نسبة الإنبات، والوزن الرطب والجاف للجذر والساق فى كلا الهجينين يقترح تأخير الإنتخاب لهذه الصفات إلى الأجيال التالية. كما يشير غياب التفوق وزيادة التباين الوراثى الإضافى لطول الساق والجذر والوزن الرطب للجذر إلى فعالية الإنتخاب فى المراحل المبكرة لهذه الصفات لتحسين تحمل الجفاف فى القمح. اوضح تحليل PCR أن اثنين من بادئات SSR واثنين من بادئات TRAP قد أظهروا اختلافات في تعدد أشكال حزم الـ DNA الناتجة. تم تحديد أربعة حزم تشير إلى تحمل الجفاف. أشار التحليل العنقودى إلى وجود ارتباط بين الطرز المتحملة للجفاف والواسمات الجزيئية المدروسة. كما أن استخدام هذه الواسمات الجزيئية فى الدراسة الحالية قد يتيح لنا الإنتخاب المبكر لتحمل الجفاف فى عشائر القمح.