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Assessment of Relationship for Both Seedling and Maturity Traits with SSR Markers under Drought Conditions in Bread Wheat (*Triticum asetivum* L.)

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TWENTY-ONE cultivars of bread wheat were evaluated for drought-stress tolerance at seedling and maturity stages under non-drought and drought-stress conditions. Significant differences among genotypes were obtained under non-drought and drought-stress conditions for all seedling and maturity characteristics. Highly positive and significant correlations were found for root length with respect to fresh weight of 0.74 and dry weight seedling of 0.80. However, negative and highly significant correlations were found for both drought susceptible index based on seedling traits (DSI_{ST}) and maturity traits (DSI_{ST}) with all seedling traits except root: shoot ratio, whereas no correlations were obtained for either DSI_{ST} or DSI_{MT} with all maturity traits except 1000 kernel weight. Positive and highly significant correlation found between DSI_{ST} and DSI_{MT} (0.85). SSR markers analysis showed that three bands produced by Xgwm596-7A (507bp), Xgwm497-1A (556bp) and Xgwm174-5D (409bp), they were presented in all tolerant genotypes based on DSI_{ST}. The three bands (507, 556 and 409bp) were correlated to DSI_{ST}, with R^2 values of 81.05%, whereas the three bands were correlated to DSI_{MT} with R^2 values of 61.96%. Strong association was observed for genotypic distance with phenotypic distance based on seedling characteristics, that amounted to 0.66, whereas the correlation was less strong between genotypic distance and phenotypic distance based on maturity traits by 0.30. The seedling traits at 15% PEG were more association than maturity traits under drought-stress with SSR markers, this gives preference to using seedling traits as an indicator of drought-stress tolerance in breeding programs.

Keywords: Bread wheat, Drought, Polymorphic marker, Seedling, SSR marker.

Introduction

Wheat (*Triticum* sp. L.) is the most widely planted crops. It supplies about 30% of the human population. Although wheat is grown in rainfed land, about 37% of the cultivated area in developing countries contains semi-arid environments (Sadok, 2017). Drought and water deficit are important abiotic stresses affecting bread wheat production worldwide. About forty-five million hectares of wheat producing land is characterized by periodic drought-stress (Byerlee & Moya, 1993). The phenotypic and genotypic assessment is the milestone to understand the genetic control of drought tolerance-related traits in wheat production programs. Polyethylene glycol (PEG) is used to induce a water deficit or drought pressure that is measured using a timescale of days after treating the seedlings with the PEG solution. Many morpho-physiological characteristics associated with drought-stress tolerance at the germination stage were used such as percentage of germination (G%) and germination pace (GP). Previous studies have recorded both traits under non-stressed and stressed conditions to estimate the decrease in G% and PG because of drought-stress (Zeng et al., 2014). Some morphological traits like shoot length (SL), root length (RL), and root: shoot ratio (R/S) can be investigated at seedling stage (Thabet et al., 2018). Significant variation was observed among bread wheat landraces for root, shoot and grain yield

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traits (Akman et al., 2017). Chaichi et al. (2019) stated that the selection for root length among landraces is possible even under drought pressure.

Estimation of the genetic diversity among germplasm sources may be increase the effectiveness of plant breeding program to improve wheat production (Barrett & Kidwell, 1998). The genetic diversity levels of evaluation among adapted genotypes can provide predictive assessments of the genetic variation among segregating progeny for pure-line cultivar development (Manjarrez-Sandoral et al., 1997), and may help portend the hybrid vigor or combining ability of the progeny in some parental combinations (Barbosa-Nato et al., 1996). DNA markers are useful tools for assessing genetic diversity among germplasm (Almanza-Pinzon et al., 2003). DNA marker-based diversity estimates reflect actual DNA differences. It uses the polymerase-chain reaction (PCR) to exponentially amplify genome segments between two specific sites (Karp et al., 1996). The genotypic data obtained from different high-density DNA markers and genome wide association study (GWAS) procedure became a common fashion for traits dissection. Many studies of GWAS have been used in wheat for complex genetic traits like grain yield, its components, and morpho-physiological traits under various environments (Shokat & Großkinsky, 2019).

Our objectives were to 1) Assess the relationship between genetic variation and phenotypic variation based on seedling or maturity traits under drought-stress conditions, 2) Determine physio-morphological traits at seedling or maturity stages that can be used by breeders to develop drought-tolerant bread wheat genotypes. 3) Compare both the relationship among maturity and seedling traits with SSR markers under drought-stress conditions, and 4) Assess the relations of different traits and grouping of cultivars according to tolerance to drought-stress.

Materials and Methods

The plant materials

The present study was carried out at the Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt during the 2019-2020 winter season. A total of twenty-one bread wheat cultivars genotypes were used (Table 1).

TABLE 1. Names and pedigree of bread wheat genotypes used in this study

Code	Name	Pedigree
G1	SAKHA 93	SAKHA 92/TR 810328
G2	SAKHA 94	OPATA/RAYON/3/JUP/BJY//URES
G3	GEMMIZA 7	CMH74.630/5X//SERI82/3/AGENT
G4	GEMMIZA 9	ALD'S'/HUAC'S'//CMH74.630/5X
G5	GEMMIZA 10	Maya 74
G6	Shandaweel1	Site//Mo/4/Nac/Th.Ac./3*Pvn/3/Mirlo/Buc
G7	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR
G8	US3-2 (LIRA SA 92)	KVZ/TRM//PTM/ANA
G9	Nour	selected early maturing inbred line (F14) derived from a cross between Shenap*Sakha69
G10	1*15	Advanced breeding line derived from inter population-antienvironmental cross between early segregates selected in two contrasting environments
G11	Line 6	Advanced long spike, short statured inbred line derived from a cross between two landraces collected from dry areas in Upper Egypt (Omara, 1994)
G12	L.S.15 (Long spike 15)	An advanced long-spike inbred line (F14) derived from a cross among landraces collected from stress areas in Upper Egypt (Omara, 1994)
G13	SIDS 1	HD2173/PAVON"S"//1158.57/MAYA 74 "S"
G14	SIDS 4	MAYA"S"/MON"S"//CMH74A.592/3/GIZA 157*2
G15	SIDS 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S" /6/MAYA/VUL//CMH74A.63014*SX
G16	Sonora 64	YAKTANA-54//NORIN-10/BREVOR/3/2*YAQUI-54
G17	DEBEIRA	HD2160/5/TOB/CNO67//BB/3/NAI60*2//TT/SN64/4/HD1954
G18	EL NIELAIN	S948.A1/7*SANTA ELENA
G19	MEXIPAK65	PENJAMO62/GABO55
G20	PAVON F 76	VCM//CNO/7C/3/KAL/BB
G21	KBG-01	300-SM-501-M/HAR-1709

Evaluation of wheat genotypes for drought tolerance at seedling stage

The polyethylene glycol (PEG6000) was used for the effects study of water stress on seedling growth parameters. The experimental design was a completely randomized block design (RCBD) with three replicates. Grains of the twenty-one genotypes were subjected to two stress level of PEG6000 i.e., 0.0% (control) and 15% (drought-stress), according to methods by Michel & Kaufmann (1973). PEG6000 was prepared by dissolving the required amount of PEG in distilled water at 30°C. Seeds of the genotypes have been disinfected using 10% sodium hypochlorite solution for five minutes, then the grains were washed three to four times with distilled water. Fifteen grains from each entry were germinated on sterilized sand in aluminum trays of 25cm wide × 50cm long × 6cm deep with respective treatments of PEG6000. The aluminum trays were covered with transparent plastic sheet to prevent the loss of moisture by evaporation under laboratory condition (24±2°C) for fourteen days. At fourteen days age, the shoot length (ShL), root length (RL), fresh weight (FW), dry weight (DW) and root/ shoot ratio (R/Sh) were estimated under control (0% PEG) and under drought-stress conditions (15% PEG). Drought susceptibility index (DSI) was estimated for all studied seedling traits, according to methods by Fischer & Maurer (1978).

Evaluation of wheat genotypes for drought-stress tolerance at maturity stage

Seeds of all entries were sown in the fields at an optimal sowing date (the 26th November). Two irrigation regimes were used as follow: 100% (favorable environment), and 50% (drought-stress environment) field water capacity in clay fertile soil at the Experimental Farm of the Faculty of Agriculture, Assiut University.

For the favorable environment, the irrigation was applied every two weeks with a total number of eight irrigations throughout the growing season. For the drought-stress environment, the irrigation was applied every four weeks with a total number of four irrigations throughout the growing season. For each environment, all genotypes were raised in a randomized complete block design (RCBD) with three replicates. Each genotype was represented in each block by ten plants per row with rows spaced 50cm apart, and plants within rows set 30cm from each other. At maturity traits, the grain yield (GY), spike length (SL), spike weight (SW), number of grains/ spike (NS/S) and 1000 grain weight (1000 KW) were estimated under favorable and drought-stress conditions. Moreover, drought susceptibility index (DSI) was estimated based on grain yield trait, according to methods by Fischer & Maurer (1978).

Molecular markers analysis

The molecular marker analysis was performed at the Department of Genetics, Faculty of Agriculture, Assiut University, Egypt. Twenty-eight SSR primer pairs were selected and used for screening the studied genotypes (Table 2). The total DNA of each cultivar was extracted according to the cetyltrimethyl ammonium bromide (CTAB) method (Murray & Thompson, 1980).

Primers sequences and PCR conditions were obtained by GrainGenes Database (http://wheat.pw.usda.gov). The PCR amplifications were performed in a SensoQuest LabCycler (SensoQuest GmbH, Göttingen, Germany). The PCR products were separated on 2.5% agarose gels in 0.5× TBE buffer. A 100bp DNA ladder was used to estimate the size of the amplified DNA fragments.

The polymorphism percentage obtained by each polymorphic marker was calculated. To investigate the suitability of each marker to assess the genetic diversity among the cultivars wheat, the polymorphic information content (PIC) was computed for each polymorphic marker using the formula described by Roldan-Ruiz et al. (2000). The marker index (MI) was computed according to Powell et al. (1996). The resolving power (Rp) of the primer was computed according to Prevost & Wilkinson (1999).

Phenotypic and molecular data analysis

The differences between means were tested by Fisher's Least Significant Difference (LSD) at 0.05 level of probability. Combined analyis of variance was performed to test the significance of differences among genotypes (G), environments (E), and the significance of G×E interaction for each character. The broad-sense heritability (h^2_B) of the studied trait was computed using the equation described by Nyquist (1991). The phenotypic correlations among the investigated traits at seedling and maturity stages were measured by Pearson's correlation coefficients.

TABLE 2. Names, chromosomal location (CL), sequences, and annealing temperature (An.) of SSR markers used in this study

Marker	CL	Forward primer	Reverse primer	An.
Xgwm33	1A	5' GGAGTCACACTTGTTTGTGCA 3'	5' CACTGCACACCTAACTACCTGC 3'	60 C°
Xgwm497	1A	5′GTAGTGAAGACAAGGGCATT–3′	5′CCGAAAGTTGGGTGATATAC–3′	55 C°
Xgwm95	2A	5' GATCAAACACACACCCCTCC 3'	5' AATGCAAAGTGAAAAACCCG 3'	60 C°
Xgwm155	3A	5' CAATCATTTCCCCCTCCC 3'	5' AATCATTGGAAATCCATATGCC 3'	60 C°
Xgwm160	4A	5' TTCAATTCAGTCTTGGCTTGG 3'	5' CTGCAGGAAAAAAAGTACACCC 3'	55 C°
Xgwm695	4A	5′AAGAGGCAGAGATGGAGTTC–3′	5′TCCCTGACACAGACGAGAT–3′	55C°
Xgwm186	5A	5' GCAGAGCCTGGTTCAAAAAG 3'	5' CGCCTCTAGCGAGAGCTATG 3'	60 C°
Xgwm459	6A	5' ATGGAGTGGTCACACTTTGAA 3'	5' AGCTTCTCTGACCAACTTCTCG 3'	55 C°
Xgwm63	7A	5' TCGACCTGATCGCCCCTA 3'	5' CGCCCTGGGTGATGAATAGT 3'	60 C°
Xgwm596	7A	5′–TGCAAAGCATCACGGAGA–3′	5′ATACACGGTGGAAGTTGGC–3′	55 C°
Xgwm260	7A	5′CACGAAGAGATATCACCCC-GAG–3′	5′GGATGTCTGCGAGCCTTTCATAT–3′	60 C°
Xgwm573	7A	5′GGGAGGCTGAGGGAATTGTC–3′	5′AGTGCCGCTGAATTCAGT-GAAA–3′	60 C°
Xgwm18	1B	5' GGTTGCTGAAGAACCTTATT-TAGG 3'	5' TGGCGCCATGATTGCATTATCTTC 3'	50 C°
Xgwm111	2B	5'GTTGCACGACCTACAAAGCA 3'	5'ATCGCTCACTCACTATCGGG 3'	55 C°
Xgwm389	3B	5' ATCATGTCGATCTCCTTGACG 3'	5' TGCCATGCACATTAGCAGAT 3'	60 C°
Xgwm513	4B	5' ATCCGTAGCACCTACTGGTCA 3'	5' GGTCTGTTCATGCCACATTG 3'	60 C°
Xgwm408	5B	5' TCGATTTATTTGGGCCACTG 3'	5' GTATAATTCGTTCACAGCACGC 3'	55 C°
Xgwm626	6B	5' GATCTAAAATGTTATTTTCTCTC 3'	5' TGACTATCAGCTAAACGTGT 3'	50 C°
Xgwm577	7B	5' ATGGCATAATTTGGTGAAATTG 3'	5' TGTTTCAAGCCCAACTTCTATT 3'	55 C°
Xgwm635	7B	5′TTGCTTGGTTGAAGGAT-TACTTC–3′	5′CCCTCGTAGGAGACCTTCTTT–3′	55C°
Xgwm458	1D	5' TTCGCAATGTTGATTTGGC 3'	5' TTCGCAATGTTGATTTGGC 3'	60 C°
Xgwm261	2D	5' CTCCCTGTACGCCTAAGGC 3'	5' CTCGCGCTACTAGCCATTG 3'	55 C°
Xgwm3	3D	5' AATATCGCATCACTATCCCA 3'	5' AATATCGCATCACTATCCCA 3'	55 C°
Xgwm165	4D	5' TGCAGTGGTCAGATGTTTCC 3'	5' CTTTTCTTTCAGATTGCGCC 3'	60 C°
Xgwm190	5D	5' GTGCTTGCTGAGCTATGAGTC 3'	5' GTGCCACGTGGTACCTTTG 3'	60 C°
Xgwm174	5D	5′TTTCTTCCGCATCAAGAGATCC–3′	5′ CCTCAGGCTATGGCACAGAAT–3′	60 C°
Xgwm325	6D	5' TTTCTTCTGTCGTTCTCTTCCC 3'	5' TTTTTACGCGTCAACGACG 3'	60 C°
Xgwm437	7D	5' GATCAAGACTTTTGTATCTCTC 3'	5' GATGTCCAACAGTTAGCTTA 3'	50 C°

Cluster analysis of wheat genotypes based phenotypic data was conducted using Standardized Euclidean Distance matrix with the unweighted pair group approach based on arithmetic averages (UPGMA) by MVSP version 3.22 software (Kovach Computing Services). The genetic distance matrix based on SSR markers was conducted and UPGMA-dendrogram was performed according to Nei and Li's coefficient using MVSP version 3.22. To assess the association between the SSR markers and studied traits, single marker analysis using linear regression was conducted by Microsoft Excel.

Results

Performance of genotypes at seedling and maturity stages under drought-stress conditions

Means performance of seedling traits under control and 15% Polyethylene glycol are presented in Table 3. Under 15 % Polyethylene glycol (15% PEG), shoot length (ShL) ranged from 3.40 (G1) to 14.41cm (G9) with an average of 8.12cm with the percent of reduction was amounted to 55.47% whereas, the root length (RL) extended 2.59 (G7) to 10.70cm (G11) with an average of 7.04cm with the reduction of 42.36%. Furthermore, the G8, G10, G11 and G12 genotypes gave maximum fresh (FW) and dry weight (DW) under 15% PEG. The reduction percentage under drought-stress conditions on 15% PEG was lower in G2, G8, G9, G10, G11, G12, G16, G18 and G20 genotypes than in other genotypes for most seedling traits (Fig. 1). Likewise, the drought susceptibility index (DSI_{ST}) estimated was less from one for G2, G8, G9, G10, G11, G12, G16, G18 and G20, indicating that these genotypes have the highest drought tolerance level about other genotypes (Fig. 2).

TABLE 3. Means of seedling traits estimated under control and 15% Polyethylene glycol (PEG) as well as drought susceptibility index (DSI)

Traits / Genotypes	ShL	RL	FW	DW	R/Sh	ShL	RL	FW	DW	R/Sh	DSI
	Control					15 % PEG					
G1	11.85	8.14	0.29	0.22	0.71	3.40	4.53	0.09	0.05	1.37	1.18
G2	16.60	13.92	0.31	0.20	0.86	5.46	8.14	0.14	0.11	1.54	0.98
G3	13.83	11.15	0.22	0.13	0.83	3.51	4.33	0.06	0.05	1.27	1.25
G4	17.20	11.54	0.22	0.14	0.69	8.34	4.74	0.06	0.04	0.58	1.19
G5	15.82	12.10	0.22	0.17	0.79	4.76	5.07	0.07	0.05	1.10	1.25
G6	16.35	10.66	0.41	0.31	0.67	7.66	5.80	0.18	0.08	0.78	1.06
G7	14.50	7.36	0.45	0.23	0.53	5.59	2.59	0.10	0.05	0.48	1.31
G8	20.54	13.21	0.41	0.31	0.66	10.63	8.98	0.21	0.17	0.87	0.80
G9	24.06	11.72	0.36	0.24	0.51	14.41	10.16	0.18	0.13	0.73	0.66
G10	18.06	10.28	0.40	0.33	0.59	9.24	8.51	0.24	0.18	0.96	0.68
G11	20.47	14.55	0.41	0.32	0.74	10.49	10.70	0.21	0.18	1.06	0.76
G12	20.41	14.50	0.36	0.30	0.74	10.26	10.36	0.21	0.16	1.05	0.76
G13	20.41	11.91	0.19	0.15	0.60	9.43	5.28	0.09	0.05	0.58	1.06
G14	13.42	11.04	0.42	0.24	0.85	7.22	4.95	0.09	0.05	0.71	1.20
G15	14.83	13.80	0.21	0.16	0.96	6.90	7.83	0.08	0.04	1.17	1.07
G16	21.15	14.73	0.37	0.30	0.72	11.51	10.16	0.18	0.15	0.92	0.81
G17	24.07	13.33	0.36	0.15	0.57	8.68	7.85	0.07	0.05	0.93	1.16
G18	20.45	14.43	0.36	0.27	0.73	10.59	8.82	0.19	0.14	0.87	0.86
G19	27.14	15.48	0.28	0.19	0.59	10.22	4.75	0.07	0.04	0.48	1.35
G20	17.42	11.82	0.29	0.19	0.70	7.67	8.61	0.13	0.09	1.16	0.86
G21	14.55	10.84	0.44	0.29	0.77	4.64	5.68	0.17	0.11	1.26	1.11
Mean	**18.24**	**12.21**	**0.33**	**0.23**	**0.71**	**8.12**	**7.04**	**0.13**	**0.09**	**0.95**	
LSD(0.05)	**1.81**	**0.98**	**0.04**	**0.03**	**0.05**	**1.31**	**1.10**	**0.03**	**0.02**	**0.13**	
% of reduction under drought stress						**55.47**	**42.36**	**59.66**	**58.91**	**-34.1**	

ShL: Shoot length, RL: Root length, FW: Fresh weight, DW: Dry weight and R/Sh: Root/ shoot ratio.

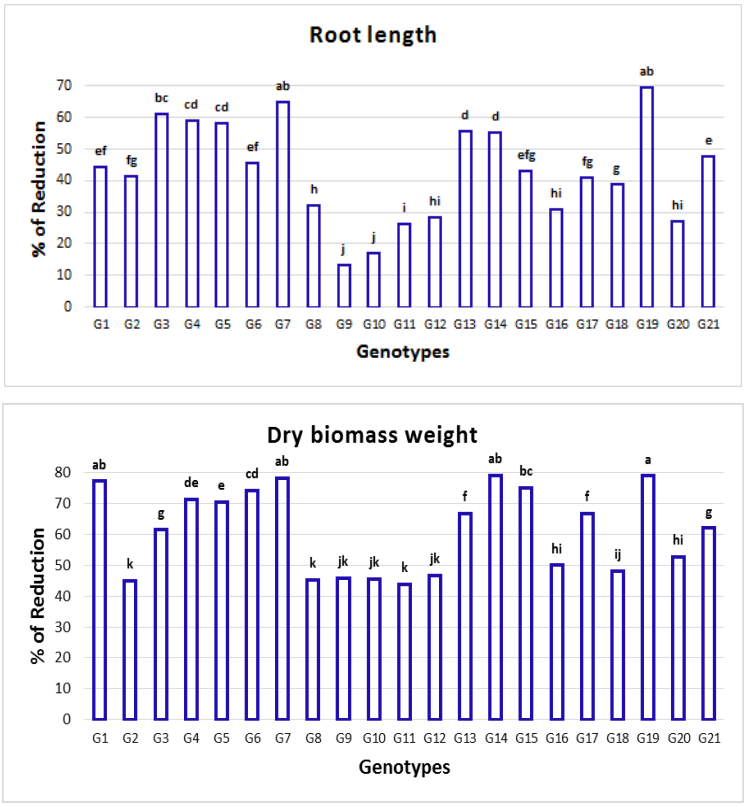


Fig. 1. The percentage of reduction in root length and dry biomass weight

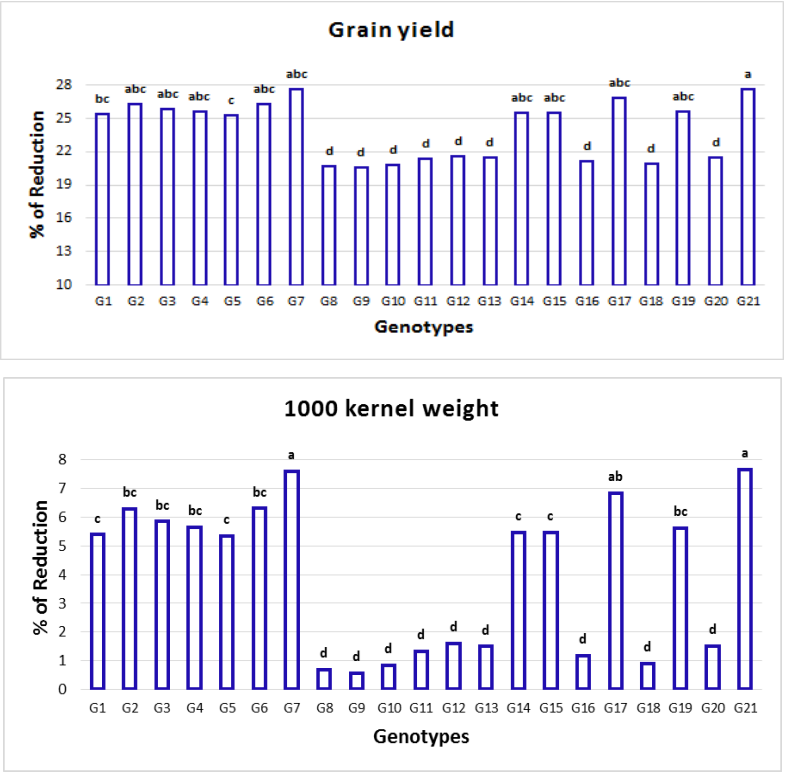


Fig. 2. The percentage of reduction in grain yield per plant and 1000 kernel weight

The maturity traits are shown in Table 4 since, the grain yield/plant (GY) ranged from 58.06 (G6) to 85.08g (G12) with an average of 68.41g whereas, the percent of GY reduction amounted 24.02% under drought-stress conditions. Also, the percent of GY reduction under drought-stress conditions was lower in G8, G9, G10, G11, G12, G13, G16, G18 and G20 genotypes compared to the remaining genotypes (Fig. 3). Similarly, under drought-stress conditions, spike length (SL), spike weight (SW), number of seeds/spike (NS/S) and 1000 kernel weight (1000KW) decreased by 13.94, 9.90, 3.98 and 3.88 % g, respectively. Finally, the G8, G9, G10, G11, G12, G13, G16, G18 and G20 genotypes have lower reduction percentage than other genotypes for 1000KW under drought-stress conditions and they have drought susceptibility index (DSI_{MT}) less from one, indicating these genotypes may be more drought tolerant (Fig. 2).

TABLE 4. Means of maturity traits estimated under favorable and drought stress as well as drought susceptibility index (DSI)

Traits / Genotypes	GY	SL	SW	NS/S	1000 KW	GY	SL	SW	NS/S	1000 KW	DSI
	Favorable					Drought stress					
G1	105.82	12.88	4.54	91.02	50.31	78.95	10.89	4.02	86.11	47.59	1.06
G2	82.97	13.61	4.06	84.06	46.22	61.15	11.39	3.56	78.76	43.31	1.10
G3	84.59	16.10	4.70	95.69	54.29	62.72	13.55	4.14	90.08	51.11	1.08
G4	89.67	14.83	4.49	90.45	45.84	66.66	12.51	3.97	85.33	43.24	1.07
G5	86.95	14.79	5.31	103.58	51.53	64.91	12.52	4.71	98.04	48.78	1.06
G6	78.78	16.04	4.20	97.90	42.52	58.06	13.43	3.68	91.72	39.84	1.10
G7	98.55	13.99	3.75	85.54	48.74	71.36	11.52	3.24	79.04	45.04	1.15
G8	73.63	10.11	3.53	67.58	54.40	58.39	9.03	3.29	67.10	54.02	0.86
G9	84.97	11.41	2.53	55.92	46.61	67.49	10.20	2.36	55.59	46.34	0.86
G10	85.32	11.73	4.44	71.94	59.21	67.55	10.46	4.14	71.34	58.71	0.87
G11	105.80	23.79	8.63	159.66	60.86	83.24	21.10	8.00	157.56	60.06	0.89
G12	108.51	25.90	8.04	146.42	55.62	85.08	22.90	7.43	144.08	54.74	0.90
G13	94.22	11.29	5.05	95.55	47.42	73.97	9.99	4.67	94.12	46.71	0.90
G14	101.67	17.13	5.84	102.86	55.71	75.77	14.48	5.17	97.23	52.66	1.06
G15	86.96	12.57	6.68	107.34	48.99	64.82	10.62	5.92	101.47	46.31	1.06
G16	83.05	12.65	4.39	95.35	45.81	65.46	11.24	4.07	94.22	45.27	0.88
G17	86.44	10.74	4.17	83.95	50.88	63.24	8.93	3.64	78.21	47.40	1.12
G18	80.50	10.59	4.34	83.64	54.70	63.67	9.43	4.04	82.89	54.21	0.87
G19	85.90	11.87	4.05	84.73	49.19	63.88	10.01	3.57	79.95	46.41	1.07
G20	86.90	15.35	3.49	84.77	39.80	68.21	13.58	3.23	83.50	39.20	0.90
G21	99.61	13.11	4.14	98.53	37.85	72.05	10.79	3.57	90.97	34.95	1.15
Mean	**90.04**	**14.31**	**4.78**	**94.59**	**49.83**	**68.41**	**12.31**	**4.31**	**90.83**	**47.90**	
LSD(0.05)	**4.41**	**1.83**	**0.67**	**10.50**	**2.71**	**3.40**	**1.63**	**0.62**	**10.40**	**2.87**	
% of reduction under drought stress						**24.02**	**13.94**	**9.90**	**3.98**	**3.88**	

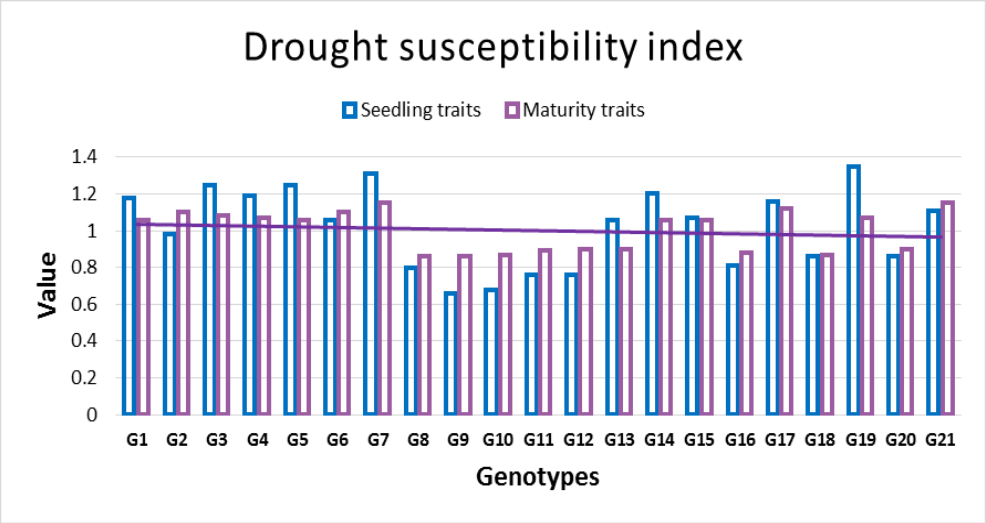


Fig. 3. Drought susceptibility values based on seedling and maturity traits

The combined ANOVA (Table 5) indicated to high significant differences were obtained between control and 15% PEG for all seedling traits. Also, high significant differences were found among wheat entries and for genotype by environment interactions for all studied seedling traits. Low to moderate estimates of heritability were detected for DW (0.43), ShL (0.38), RL (0.23), FW (0.21) and R/Sh ratio (0.12). Otherwise, high significant differences were obtained among environments and genotypes for all maturity traits while, the genotype environment interaction estimated was significant only in GY and SL traits. In addition, moderate to high values of heritability were observed and ranged from 0.59 for GY to 0.94 for SW (Table 6).

Phenotypic correlations among studied traits under drought-stress

Correlation coefficients among all studied traits at seedling and maturity stages under drought-stress, are shown in Table 7. Among seedling traits, highly significant and positive correlation coefficients were found for ShL with RL (0.68), FW (0.54) and DW (0.51), while negative correlation was identified for ShL with R/Sh ratio (-0.51, $P<0.01$). Likewise, highly significant, and positive correlations were found for RL with FW (0.74) and DW (0.80), also between FW and DW (0.96). From the other side, among maturity traits, high significant positive correlations were obtained for GY with SL (0.67), SW (0.66) and NS/S (0.68), for SL with SW (0.79) and NS/S (0.87) and for SW with NS/S (0.94) and 1000KW (0.55). Finally, no correlation found between maturity and seedling traits except that found between DW and 1000 KW (0.48, $P<0.01$). However, negative, and highly significant correlations were found for both drought susceptible index based on seedling traits (DSI_{ST}) or maturity traits (DSI_{ST}) with all seedling traits except RL/ShL ratio Whereas no correlations were obtained for either DSI_{ST} or DSI_{MT} with all maturity traits except 1000KW. Also, positive and highly significant correlation was found between DSI_{ST} and DSI_{MT} (0.85).

SSR markers

Out of twenty-eight SSR primers used for screening twenty-one of bread wheat genotypes, nine polymorphic primers were obtained, they generated forty-six bands, which ranged from three bands for Xgwm160-4A, and Xgwm573-7A to 8 bands for Xgwm497-1A, with an average of 5.11 bands per polymorphic primer. Of forty-six bands generated, seventeen bands were polymorphic by average value 1.89 bands/primer. The lowest polymorphism (20%) was observed with Xgwm174-5D, whereas the highest polymorphism (66.7%) was produced by two SSRs, with 40.59% averaged polymorphism. The polymorphism information content (PIC) values ranged from 0.21 for Xgwm635-7B and Xgwm573-7A to 0.49 for Xgwm174-5D, with an average of 0.34. The highest MI value (0.86) was obtained for Xgwm260-7A and the lowest MI value (0.42) was observed in Xgwm573-7A (Table 8).

TABLE 5. Combined analysis of variance for maturity traits as well as broad sense heritability

Maturity traits							
Favorable				Drought stress			
S. O. V	Rep	Gen. (G)	Error	S. O. V	Rep	Gen. (G)	Error
df	2	20	40	df	2	20	40
GY	7.75	281.7**	10.12	GY	4.42	167.5**	6.23
SL	0.22	48.64**	0.28	SL	0.17	38.6**	0.22
SW	0.05	6.41**	0.03	SW	0.04	5.6**	0.03
NS/S	14.24	1597.4**	11.90	NS/S	13.7	1565.1**	11.55
1000KW	2.19	106.2**	3.13	1000KW	1.99	119.7**	3.02

Combined						
S. O. V	Tr. (T)	Rep./En.	Gen. (G)	G x T	Error	Heritability (B.S.)
df	1	4	20	20	80	
GY	14736.8**	6.09	430.85**	18.35**	7.98	0.59
SL	125.31**	0.2	86.71**	0.51*	0.25	0.91
SW	7.06**	0.04	11.91**	0.04	0.03	0.94
NS/S	447.29**	13.95	3153.6**	8.83	11.37	0.93
1000KW	117.72**	2.09	223.5**	2.3	3.2	0.79

GY: Grain yield, SL: Spike length, SW: Spike weight, NG/S: Number of grains/ spike and 1000KW: 1000-grain weight.

TABLE 6. Combined analysis of variance for seedling traits as well as broad sense heritability

Seedling traits							
Control				15 % PEG			
S. O. V	Rep	Gen. (G)	Error	S. O. V	Rep	Gen. (G)	Error
df	2	20	40	df	2	20	40
ShL	0.47	47.52**	0.44	ShL	0.13	24.67**	0.09
RL	0.23	13.80**	0.19	RL	0.11	17.50**	0.07
FW	0.11	21.30**	0.15	FW	0.03	10.63**	0.03
DW	0.07	13.03**	0.07	DW	0.02	8.26**	0.01
R/Sh ratio	0.42	40.58**	0.62	R/Sh ratio	0.05	25.87**	0.11

Combined						
S. O. V	Tr. (T)	Rep./En.	Gen. (G)	G x T	Error	Heritability (B.S.)
df	1	4	20	20	80	
ShL	3225.72**	0.3	64.14**	8.05**	0.25	0.38
RL	842.93**	0.17	25.36**	5.95**	0.12	0.23
FW	1238.60**	0.07	25.56**	6.37**	0.09	0.21
DW	579.83**	0.04	19.24**	2.05**	0.04	0.43
R/Sh ratio	182.60**	0.05	21.37**	8.55**	0.09	0.12

ShL: Shoot length, RL: Root length, FW: Fresh weight, DW: Dry weight and R/Sh: Root/ shoot ratio.

SSR markers analysis revealed that three bands generated by Xgwm596-7A (507bp), Xgwm497-1A (556bp) and Xgwm174-5D (409bp) (Fig. 4). SSR markers were presented in G2, G8, G9, G10, G11, G12, G13, G16, G18 and G20 genotypes. The three bands (507, 556 and 409bp) were correlated to DSI_{ST}, with R^2 values of 81.05% at seedling traits, whereas the three bands were correlated with DSI_{MT} with R^2 values of 61.96 (Table 9 and Fig. 4).

Cluster analysis, which was performed based on the seedling traits data separated the investigated genotypes into two sub clusters. The first cluster contained all the tolerant genotypes except G2 and G20, while the second cluster contained the remaining genotypes. Likewise, the cluster analysis based on the molecular marker data divided the investigated genotypes into two sub clusters. The first cluster consisted of all the drought of tolerant genotypes and the second contained all the non-

tolerant genotypes. Whereas the dendrogram of the maturity traits divided the genotypes into three sub clusters. The first and second clusters contained all the drought of tolerant genotypes, while the third cluster contained the remaining of genotypes. These results indicating that the cluster analysis of the seedling traits was more similar with the cluster analysis based on molecular analysis than the cluster analysis based on maturity traits (Fig. 5).

Positive and high significant correlation was identified between genotypic distance and phenotypic distance based on seedling or maturity traits (Fig. 4). Strong correlation was determined for genotypic distance with phenotypic distance based on seedling traits which amounted 0.66, whereas the correlation was less strong between genotypic distance and phenotypic distance based on maturity traits by 0.30 (Fig. 6).

TABLE 7. Correlation coefficient among all studied traits at seedling and maturity stages under drought stress

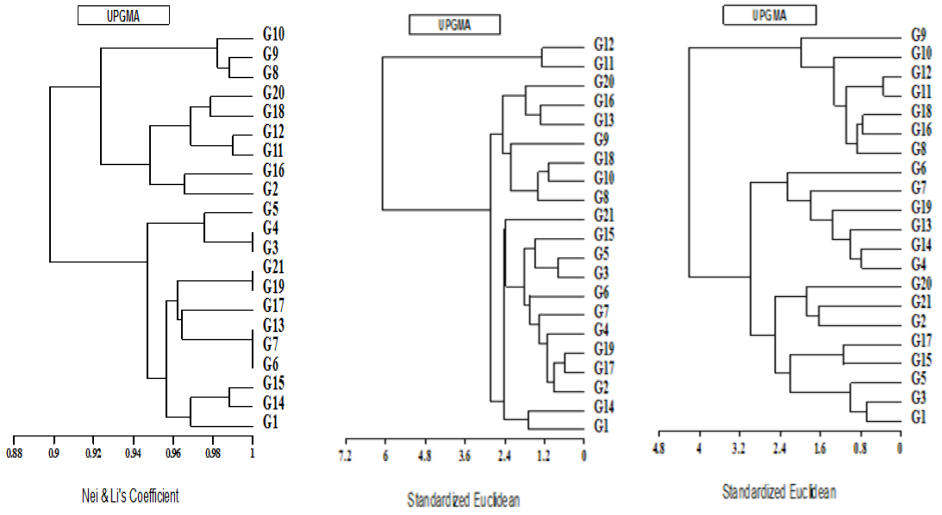
Traits	ShL	RL	FW	DW	R/Sh ratio	GY	SL	SW	NS/S	1000 KW	DSI (ST)
RL	0.68**										
FW	0.51**	0.72**									
DW	0.54**	0.80**	0.96**								
R/Sh ratio	-0.51**	0.25	0.11	0.18							
GY	0.00	0.09	0.15	0.18	0.05						
SL	0.04	0.28	0.29	0.30	0.16	0.67**					
SW	0.04	0.30	0.19	0.23	0.17	0.66**	0.79**				
NS/S	-0.01	0.25	0.19	0.21	0.19	0.68**	0.87**	0.94**			
1000KW	0.32	0.36	0.36	0.48**	-0.05	0.31	0.35	0.55**	0.35		
DSI (ST)	-0.67**	-0.90**	-0.86**	-0.89**	-0.16	-0.14	-0.23	-0.17	-0.12	-0.38*	
DSI (MT)	-0.73**	-0.74**	-0.64**	-0.72**	0.10	-0.17	-0.16	-0.19	-0.11	-0.52**	0.85**

ShL: Shoot Length, RL: Root Length, FW: Fresh weight, DW: Dry weight and R/Sh: Root /shoot ratio, GY: Grain yield, SL: Spike length, SW: Spike weight, NG/S: Number of grains/ spike and 1000KW: 1000-grain weight.

TABLE 8. Number of total bands, polymorphic bands, PIC, MI and RP for each polymorphic of SSR primer

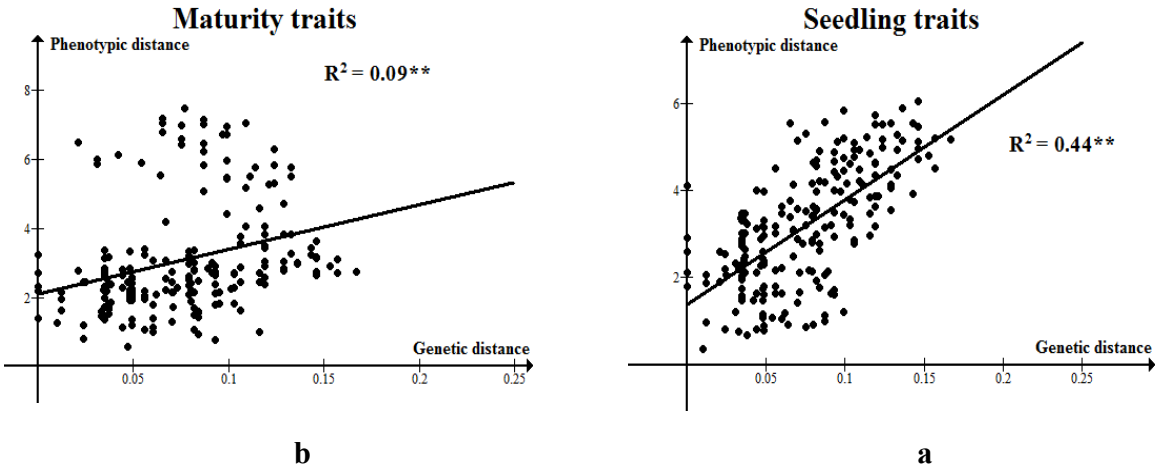
Markers	TAB	NPB	%PB	PIC	MI	RP
Xgwm160-4A	3	2	66.7	0.25	0.50	1.33
Xgwm577-7B	4	1	25.0	0.47	0.47	0.76
Xgwm695-4A	6	2	33.3	0.25	0.50	3.43
Xgwm596-7A	7	2	28.6	0.37	0.74	2.57
Xgwm497-1A	8	2	25.0	0.34	0.68	1.43
Xgwm260-7A	5	2	40.0	0.43	0.86	1.90
Xgwm174-5D	5	1	20.0	0.49	0.49	0.86
Xgwm635-7B	5	3	60.0	0.21	0.63	5.52
Xgwm573-7A	3	2	66.7	0.21	0.42	1.91
Average	**5.11**	**1.88**	**40.59**	**0.34**	**0.59**	**2.19**

TAB: Total amplified bands, NPB: No. of Polymorphic bands, %PB: % of Polymorphism, PIC: Polymorphic information content, MI: Marker index, RP: Resolving power.



a b c

Fig. 4. Dendrogram of 21 wheat genotypes developed from seedling data (a), maturity data (b) and SSR marker data (c) using UPGMA analysis



b a

Fig. 5. Correlation of genetic distance with phenotypic distance based on seedling data (a) and maturity data (b)

Discussion

The selection and development of drought tolerant genotypes that maintain productivity in semiarid environments will be critical for ensuring adequate food supplies in the future (Foley et al., 2011). Although drought-stress may influence wheat at any stage in development, it is particularly injuring dangerous during seedling growth (Pessarakli, 2016), because it may cause early senescence and finally plant death (Wang et al., 2015). The seedling stage determines both structure and dynamics of the advanced growth stages for most crop populations (De La Cruz et al., 2008), because the vegetative stage affects the economic yield at the final stage of growth, and the photosynthetic reserves accumulated until the flowering provides 57% of the resultant grain yield (Gallagher et al., 1976).

In the present study, we evaluated twenty-one genotypes for drought-stress tolerance at both seedling and maturity stages. At seedling stage, the root length, shoot length, fresh weight and dry weight were reduction under 15% polyethylene glycol (PEG) by 55.47, 42.36, 59.66, 58.91%, respectively. At the maturity traits, the percent reductions were obtained under drought-stress conditions by 24.02, 13.94, 9.90, 3.98 and 3.88% for grain yield per plant (GY), spike length (SL),

spike weight (SW), number of grains per spike (NG/S) and 1000 kernel weight (1000KW), respectively. These results agree with Dhanda et al. (2004). The lowest percent of reductions under drought at maturity and seedling traits were obtained for G8, G9, G10, G11, G12, G16, G18 and G20 tested genotypes, also they have drought susceptibility index (DSI) values less from one, indicating that these genotypes have the highest drought tolerance level about other genotypes. Many scientists reported that drought resistance is considered by small reduction of dry weights under water-stress environments (Ahmed et al., 2019). Sareen et al. (2014) found that nine tolerant wheat landraces based on drought susceptibility index in a study that evaluated twenty-one genotypes. Becker et al. (2016) reported that one of the synthetic hexaploid wheat lines was shown to be superior under drought conditions for root morphological traits including root deep and length.

In the current study, the combined ANOVA revealed highly significant differences among genotypes under non-drought and drought-stressed conditions for all seedling and maturity traits. Also, high significant differences were obtained genotype by environment interactions for all studied traits. Moreover, low, and moderate values of broad-sense heritability were estimated for seedling traits, while moderate to high broad sense heritability were obtained for maturity traits. All investigated traits showed remarkable variations in water deficit environments at seedling stage (Ahmed et al., 2020). El-Rawy & Hassan (2014) obtained low to moderate estimates of narrow-sense heritability for root length, shoot length and seedling dry weight at 15% PEG. Dhanda et al. (2004) observed considerable genetic differences for all investigated traits except shoot length under drought-stress, they also obtained moderate to high heritability estimates for all investigated traits under drought-stress. The estimates of broad sense heritability ranged from 0.34 to 0.99 for seedling traits which proposed that the selection for these traits will be effective (Khan et al., 2002).

Under drought-stress conditions, the correlation of most of the seedling or maturity traits were positive and significant with each other in this study. Also, high significant and negative correlation were found for drought susceptibility index based on maturity traits (DSI_{MT}) with seedling traits, whereas no correlation was identified between drought susceptibility index based on seedling traits (DSI_{ST}) and maturity traits except 1000KW. These previous results indicate to evaluation for drought-stress tolerance based on seedling traits may more effective than evaluation based on maturity traits. Ahmed et al. (2020) observed that most of the seedling traits were positive and significant correlation with each other. Grain yield per spike was significantly associated to root length (r= 0.41) and seedling dry weight (r= 0.46) at 15% PEG (El-Rawy & Hassan, 2014). A significant positive association was observed between cell membrane stability and DTI (Geravandi et al., 2011).

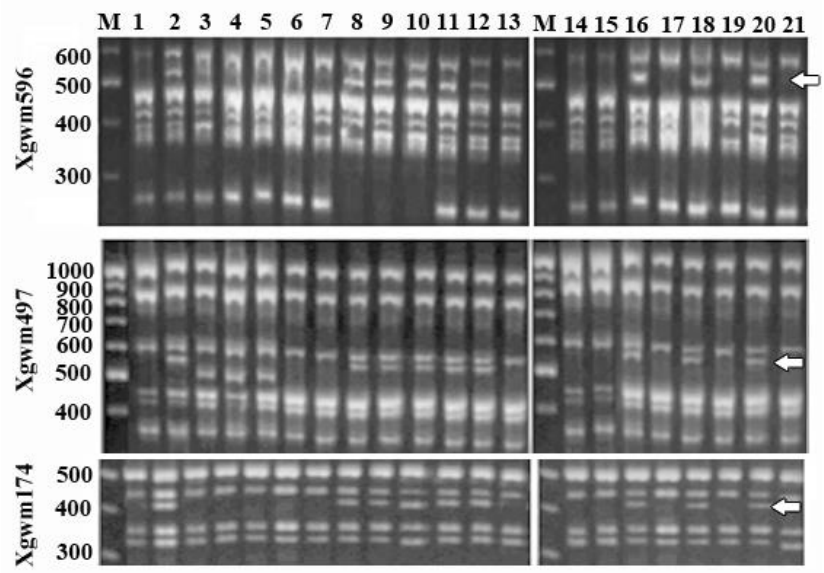


Fig. 6. PCR amplification patterns obtained using Xgwm 596, Xgwm 497 and Xgwm 174 SSR markers in twenty-one studied genotypes [M: A 100bp DNA ladder]

In this study, twenty-eight SSR primers used for screening twenty-one bread wheat genotypes, nine polymorphic primers were obtained, they generated forty-six bands by average value 5.11 bands per polymorphic primer. Of forty-six bands generated, seventeen bands were polymorphic by average of 1.89 bands/ primer. PIC values varied from 0.21 for Xgwm635-7B and Xgwm573-7A to 0.49 for Xgwm174-5D, by average value 0.34. SSR markers analysis revealed three bands generated by Xgwm596-7A (507bp), Xgwm497-1A (556bp) and Xgwm174-5D (409bp) SSR markers, they were presented in all genotypes which have DSI_{ST} values less than from one, while they not found in all genotypes which have DSI_{MT} values less than from one. Moreover, three bands (507, 556 and 409bp) were correlated with DSI_{ST}, with R^2 values of 81.05%, whereas the three bands were associated with DSI_{MT} with R^2 values of 61.96. Strong correlation was identified for genotypic distance with phenotypic distance based on seedling traits, whereas the correlation was less strong between genotypic distance and phenotypic distance based on maturity traits. These previous results indicate to the seedling traits at 15% PEG were more association than maturity traits under drought-stress with SSR markers.

Many previous studies have detected DNA loci in wheat correlated to different morpho-physiological traits under drought-stress conditions like carbon isotope discrimination that mapped on 1BL, 2BS, 3BS, 4AS, 4BS, 5AS, 7AS, and 7BS (Rebetzke et al., 2008), seedling vigor mapped on 6A (Spielmeyer et al., 2007) and coleoptile length located on chromosomes 4B and 6A (Rebetzke et al., 2001). Tura et al. (2020) identified QTLs for yield under drought-stress in a doubled haploid (DH) population in wheat on 4A, 5B, and 7A. In wheat, Touzy et al. (2019) detected 24, 31, and 28 QTL correlated to low, medium and high drought-stress tolerance, respectively. Bhatta et al. (2018) determined ninety marker-trait associations (MTAs) related to grain yield and related traits under water deficit conditions. Likewise, QTLs for drought-related measures like normalized difference vegetative index (NDVI), drought susceptibility index (DSI), and leaf traits (including leaf senescence, green leaf area, and flag leaf phenotypes) were mapped on chromosomes 1B, 4A, 6B, 5B, 7A, and 7B (Edae et al., 2014) in spring wheat. Two markers mapped on 1A and 2D were correlated with plant height, while spikelets/ spike was highly correlated to eight markers located on 6B, 2D, 2B, 5D, 1B and 4B Under drought-stress, (Mwadzingeni et al., 2017). González et al. (2020) observed association between five root system architecture variables and SSR markers. Sallam et al. (2019) stated that genotypes may be characterized as tolerant to drought at the germination or seedling stage, but these genotypes may be very sensitive to drought at the flowering stage. Therefore it would be better to test genotypes at different growth stages. Drought-stress tolerance is a multigenic trait governed by several genes with minor effects (Bernardo, 2008).

Conclusion

Twenty-one genotypes evaluated for drought tolerance at seedling and maturity stages. Highly significant differences among genotypes were obtained under non-drought and drought-stress conditions for all seedling and maturity traits. Under drought condition, the correlation of most of the seedling or maturity traits were positive and significant with each other. Furthermore, high significant and negative correlation were found for DSI_{MT} with seedling traits, whereas no correlation was found between DSI_{ST} and maturity traits except 1000 KW. SSR markers analysis revealed that three bands generated by Xgwm596-7A (507bp), Xgwm497-1A (556bp) and Xgwm174-5D (409bp), they were presented in all tolerant genotypes based on DSI_{ST}. The seedling traits at 15% PEG were more associated than maturity traits under drought-stress with SSR markers, giving preference to using seedling traits as an indicator of drought tolerance in drought tolerance breeding programs.

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تقدير العلاقة بين كل من صفات البادرات والنضج مع واسمات الـ SSR تحت ظروف الجفاف في قمح الخبز (*Triticum asetivum* L)

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تم تقييم واحد وعشرون تركيب وراثي من قمح الخبز لتحمل إجهاد الجفاف في مرحلتي البادرات والنضج تحت كل من الظروف المواتية وظروف الجفاف. تم الحصول على فروق معنوية بين التراكيب الوراثية تحت كل من الظروف المواتية وظروف اجهادات الجفاف لجميع صفات البادرات والنضج. وجد ارتباط موجب وعالي المعنوية لطول الجذر مع كل من الوزن الرطب (0.74) والوزن الجاف للبادرات (0.80). بينما وجدت ارتباطات سلبية وعالية المعنوية لكل من مؤشر الحساسية للجفاف بناءً على صفات البادرات (DSI_{ST}) وصفات النضج (DSI_{MT}) مع جميع صفات البادرات باستثناء نسبة الجذرإلى الساق، في حين لم يتم الحصول على أي ارتباطات لكل من DSI_{ST} أو DSI_{MT} مع صفات النضج باستثناء وزن 1000 حبة. تم الحصول على ارتباط موجب وعالي المعنوية بين كل من DSI_{ST} وDSI_{MT} (0.85). أظهر تحليل واسمات SSR أن ثلاثة شظايا نتجت بواسطة البادئات (507 bp) Xgwm596-7A و(556 bp) Xgwm497-1A و(409 bp) Xgwm174-5D تم مشاهدتها في جميع الأنماط الجينية المتحملة بناءً على DSI_{ST}. ارتبطت الشظايا الثلاثة (409, 556 , 507 bp) بـ DSI_{ST} بقيمة R^2 بلغت 81.05%، في حين ارتبطت الشظايا الثلاثة بـ DSI_{MT} بقيمة R^2 بلغت 61.96%. وجد ارتباط قوي بين البعد الوراثي والبعد المظهري بناءً على صفات البادرات التي قدرت 0.66، بينما كان الارتباط أقل قوة بين البعد الوراثي والبعد المظهري بناءً على صفات النضج بمقدار 0.30. كانت صفات البادرات تحت 15% من PEG أكثر ارتباطًا من صفات النضج تحت اجهاد الجفاف مع واسمات SSR، وهذا يعطي الأفضلية في استخدام صفات البادرات كمؤشر على تحمل اجهادات الجفاف في برامج التربية.