Egypt. J. Plant Breed. 23(1):77– 92 (2019)

SELECTING DIVERSE BREAD WHEAT GENOTYPES UNDER SALINE STRESS CONDITIONS USING ISSR MARKER AND TOLERANCE INDICES

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ABSTRACT

The study aimed at identifying and assessing salt tolerance and genetic diversity of five wheat genotypes in two diverse locations (Moshtohor and Siwa) during the winter season of 2016/2017. A total of 46 amplified fragments were generated by the five primers, 54.35% of them were polymorphic. High polymorphism (63.64%) was observed by using primer 17899A. 7 and 11 unique bands were detected in positive and negative states, respectively. These bands distinguished the genotypes for their tolerance to stress. Highest genetic diversity of 40.5% was obtained between Shandweel-1 and Misr-1 genotypes, whereas, the lowest one of 20.4% was between Nesr and S8/17 genotypes. Total number of effective alleles was 82.34. The polymorphic information content per primer ranged from 0.885 for 17898B primer to 0.959 for 17899B primer with an average of 0.922. Grains yield m^{-2} was significantly different between the stress conditions (Siwa) and normal conditions (Moshtohor). Differences varied among wheat genotypes. The highest grain yield by S8/17 (S8 tissue culture regenerated doubled haploid line) followed by Misr-1. Stress tolerance index (STI) and grain yield were the highest for line S8/17 and the lowest for Misr-2. Similar ranks for such genotypes were observed by geometric mean productivity (GMP) and Harmonic mean (HM) parameters as well as for yield index (YI). Nesr genotype which showed the highest stress tolerance (TOL) and stress susceptibility index (SSI) as well as yield stability index (YSI) suggests more stress tolerance mechanisms in this genotype.

Key words: ***Bread wheat, Cluster analysis, Genotypes, ISSR marker, Siwa Oasis, Stress tolerance indices.***

INTRODUCTION

Wheat is the vital crop in the world (Al Saadoown *et al* 2018). Egypt imports about 45% of its wheat requirements. Demand for wheat increased considerably with restriction of its available production resources. This requires more efforts to raise wheat production. Thus, a need to improve varieties with wider adaptability and stable performance through crop improvement programs is detected. During recent years increasing wheat production under certain abiotic stress conditions has become very important. The most important of these problems are salinity, which has negative effects on plant and production (Metwaly 2012). Selection of genotypes under environmental stress condition is one of the main tasks of plant breeders for exploiting the genetic variations to obtain the most tolerant cultivars (Clarke *et al* 1984 and1992). Various quantitative criteria have been proposed for selection of genotypes based on their yield, and tolerance to stress environments. Based on these indicators, genotypes are compared under stress and non-stress conditions (Taghian and Abo-Elwafa 2003).

Siwa Oasis is located at west of the Qattara Depression and its soils are mainly saline. It depresses about 25 m below sea level. According to Madani (2005) soil salinity is due to saline irrigation water, the ground water is located near the surface, 1.2 m also; salts accumulate in the topsoil as a result of evaporation.

Stress resistance is defined by Hall (1993) as the relative yield of genotype compared with other genotypes subjected to the same stress. Stress susceptibility of a genotype is often measured as a function of the reduction in characteristic performance under stress (Bidinger *et al* 1978). Some selection indices have been proposed based on a mathematical relation between stress and adequate conditions. Utilization of the selection indices is to evaluate response of plant genotype to abiotic stress and provide a measure of injury based on loss of characteristic performance under stress conditions in comparison with normal conditions (Mitra 2001). These indices are either based on stress resistance or on susceptibility of the genotype (Fernandez 1992).

Presence of genetic diversity and genetic relationships among genotypes is a prerequisite and of a paramount importance for a successful wheat breeding program. Developing wheat varieties with desirable traits requires knowledge about the existing genetic variability (Singhal and Upadhyay 1977, Maniee *et al* 2009, Kahrizi *et al* 2010 and El-Hosary and Nour El Deen 2015).).

The DNA molecular markers which are based on PCR (such as inter simple sequence repeats "ISSRs") have become excellent tools for plant breeders to select the genetic materials that are tolerant to stresses, regardless of any interaction with the environment.

Accordingly, the present study was undertaken to study grain yield and crop components of five bread wheat genotypes across two diverse environments to screen tolerance criteria and select the more adaptable genotypes under salinity stress.

MATERIALS AND METHODS

Plant materials

This study was conducted on three recently released Egyptian bread wheat (*Triticum aestivum*) cultivars (Misr-1, Misr-2 and Shandweel-1) obtained from Agricultural Research Center, the bread introduced line (Nesr) and S_8 double-haploid line (S8/17) was obtained as somaclones *via* regeneration of plants from callus derived from immature inflorescences / embryos of Egyptian hexaploid bread wheat Sakha 8. The tissue culture regenerated doubled haploid plant is termed R_0 generation and subsequent generations up to S_8 selfed progeny for this line. The code, pedigree and or selection history of all aforementioned genotypes are presented in Table (1).

Table 1. Origin, pedigree and selection history of the five bread wheat cultivars and lines used in the present study

No.	Genotype	Origin	Pedigree and/or selection history
G.1	Misr-1	Egypt	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOOYO 1881T-050M-030Y-030M-030WGY-33M-0Y-0S.
G.2	Misr-2	Egypt	SKAUZ/BAV 92. CMSS96M03611 S-1M-010SY-010M-010SY-8M-0Y-0S.
G.3	Shandaweel-1	Egypt	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH.
G.4	Nesr	CIMMYT/ ICARDA#	ICW85-0024-06AP-300AP-300L-1AP-0AP
G.5	S8 / 17*	Egypt	R8 tissue culture regenerated double haploid plant

CIMMYT: Centro International de Mejoramiento de Maize Y Trigo (Mexico) = International maize and wheat improvement center.
 # ICARDA: International Center for Agricultural Research in the Dry Areas.
*** Newlythe bred lines released through Desert Research Center (wheat breeding program).**

DNA extraction and ISSR-PCR technique

Extraction of DNA was done from all genotypes using mi-plant genomic DNA Isolation Kit (Metabion) after collecting young leaf tissues from each genotype and bulked, lyophilized and ground with a mortar. Then the DNA was determined for each stock of samples and diluted to a uniform concentration of 10 ng μL^{-1} to be used with PCR marker. ISSR reaction was conducted using five short (10-20 base-pairs) stretches of DNA that are hyper variable primers, their sequences are shown in Table (2). Reactions were carried out in a total volume of 25 µL containing 30 ng of genomic DNA as a template, 30 pmoles of random primer, 2mM of dNTP's mix (dATP, dCTP, dTTP and dGTP, ABgene, Surrey, UK), 10 X PCR buffer, 25 mM $MgCl_2$, and 2 units Taq DNA polymerase (promega, USA) (Weising *et al* 2005).

Table 2. ISSR primers codes and sequences used in PCR analysis.

No.	Name	Sequence (5`→ 3`)
1	844 B	$(CT)_8$ AC
2	17899 B	$(CA)_6$ GG
3	17898 B	$(CA)_6$ GT
4	HB 2	$(CAG)_5$
5	17899 A	$(CA)_6$ AG

Amplifications were carried out in a thermo cycler (UNO II Biometra) programed for 94ºC For 4 min, (one cycle); followed by 94ºC for 45 sec, 38ºC for 1 min, and 72ºC for 1 min (35 cycle); 72ºC for 10(one cycle) ,then 4ºC (infinitive).

The amplification products were resolved by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 µg mL^{-1}), visualized with ultraviolet light and photographed. DNA fragment sizes were determined by comparison with the 100bp and 1Kb DNA ladder marker (promega USA). Bands were detected on UV-transilluminator and photographed by Gel documentation system Biometra Bio Doc Analyzer 2000.

Concerning the potential characterization of different systems of molecular markers to evaluate the inter-populational variability to the studied genotypes, different parameters were calculated, i.e. total polymorphism generated by a certain primer polymorphic information content (PlC) indicated its discriminatory power:

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}^2 - \sum_{i=1}^{n=1} \sum_{j=i+1}^{n} 2P_i^2 - P_j^2$$

Where, Pi, Pj, Pij, i and j refer to allele i frequency, locus frequency, allele frequency for locus, n- total number of loci, respectively.

To test the efficiency of a certain primer in polymorphism detection discrimination index (Pi) was calculated according to Dangi *et al* (2004) as follows.

$$PI = \sum PIC$$

Genetic similarity coefficients between a pair of genotypes were produced for the ISSR data using Nei and Li's formula (1979). A dendrogram tree was constructed by the UPGMA clustering algorithm from the SAHN option of NTSYS-PC version 2.1(Rohlf 2000). The discriminative power (Dj= 1-Cj) and confusion probability (Cj) of the jth assay (Tessier *et al* 1999) were calculated according to the following equation: Cj= $\sum^{I}{}_{i=1}$ pi (Npi - 1)/N-1, where, pi represents the frequency of the i^{th} pattern, N the sample size, and I is the total number of patterns generated by the j^{th} assay.

Field traits

The five bread wheat genotypes were grown during the season 2016/207 at Siwa Oasis, Tegzerty Experimental farm of Desert Research Center 320 km from Marsa Matrouh (29°12"N, 26°3" E) as a salinity stressed environment. Also, the same genotypes were grown during the same season in Kalubia Governorate, at the Experiment and Research Station of Moshtohor Faculty of Agriculture, Benha University, Egypt as a non-salilnity environment.

The Siwa Oasis soil of the experimental site was a saline sandy loam (EC 12.3 dSm^{-1}) calcareous (175g $CaCO_3$ kg^{-1}). Irrigation water was a saline well water with EC of 3.96 $dSm^{-1}$and pH 7.3. The moshtohor soil was a non-saline (EC=1.8 dsm^{-1}) non-calcareous and irrigated with a non-saline Nile water. Main properties of soil and irrigation water are presented in Table (3 a and b). Physical and chemical analysis of soil and irrigation water were determined according to Jackson (1960) and Piper (1947).

Table 3 a. Soil physical analysis of the two experimental sites at Siwa Oasis and Moshtohor locations during 2016/2017 growing season.

Location	Mechanical Analysis (%)				Texture
	Course sand	Fine sand	Silt	Clay	
Siwa Oasis	9.8	12.4	60.82	16.98	Sandy loam
Moshtohor	6.5	27.5	12.4	53.6	Clay

Table 3 b. Chemical analysis of soil and irrigation water during 2016/2017 growing season.

Location	OM g kg^{-1}	$CaCO_3$ g kg^{-1}	pH	EC (dSm^{-1})	Soluble anions (meq/L.)			Soluble cations (meq/L.)			
					Cl^-	HCO_3^-	SO_4^{--}	Na^+	K^+	Ca^{2+}	Mg^{2+}
Soil analysis (0 – 30cm)											
Siwa Oasis	53	17.5	7.9	12.3	83.6	2.3	36.1	68.9	1.6	34.5	17.4
Moshtohor	21	2.8	8.0	0.5	9.8	1.1	7.1	8.7	0.4	5.7	3.2
Irrigation water analysis											
Siwa Oasis	None	None	7.3	3.96	18.6	10.8	8.0	20.1	0.5	8.2	8.7

Notes: pH of 1:2.5 with water suspension for soil.

Grains were sown on the last week of Nov., 2016. The experimental design was a randomized complete blocks with three replications. The plot size was 10.5 m^2 (3.0 x 3.5 m). Grains were sown in rows 20 cm apart. Agricultural practices recommended for growing wheat were followed in each of the two locations.

Data for the following traits were recorded on square meter in each plot for number of spikes/ m^2 and number of grains per main spike, 1000-kernel weight and grain yield/m^2.

Statistical analysis

Duncan's multiple range test (Duncan 1955) was used to verify the significance of mean performances for all yield traits recorded in both and across locations.

The calculation of the tolerance indices:

1. Tolerance index (TOL) and mean productivity (MP) as done by Rosielle and Hamblin (1981):

TOL = ($Y_p - Y_s$) and MP = ($Y_s + Y_p$)/2

2. Harmonic mean (HM) (Kristin *et al* 1997):

HM = 2($Y_p * Y_s$) / ($Y_p + Y_s$)

3. Stress susceptibility index (SSI) (Fisher and Maurer 1978):

SSI = 1 – (Y_s / Y_p) / SI, while SI = 1 – ($\hat{Y}_s / \hat{Y}_p$)

Whereas SI is stress intensity and $\hat{Y}s$ and $\hat{Y}p$ are the means of all genotypes under stress and normal conditions, respectively.

4. Geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez 1992 and Kristin *et al* 1997):

GMP = $(Y_p * Y_s)^{½}$ STI = ($Y_p * Y_s$) / $(\hat{y}_p)^2$

5. Yield Index (YI) (Gavuzzi *et al* 1997 and Lin *et al* 1986):

YI = $Y_s / \hat{Y}_s$

6. Yield Stability Index (YSI) (Bouslama and Schapaugh 1984):

YSI = Y_s / Y_p

RESULTS AND DISCUSSION

Inter Simple Sequence Repeats (ISSR)

Results of the DNA of the five bread wheat genotypes which were subjected to PCR against five ISSR primers are illustrated in Figure 1 and Tables (4). A total of 46 amplified fragments were generated by the five primers, out of them 25 were polymorphic (54.35%). The number of amplicons per primer varied from 7 for each of 844 B and 17898 B to 12 for HB 2 primer. The size of the amplified fragments ranged from 120 bp (AF16) to 2875 bp (AF01). High polymorphism was detected by using primer 17899A and reached 63.64%. This primer scored seven polymorphic amplicons out of 11 TAF (Table 5). Primer 844 B produced 7 bands in which fragment sizes ranged from 2875 to 1104 bp, four of them were polymorphic(57.14% polymorphism). Primer 17899 B produced 9 fragments with sizes ranging from 614 to 120 bp, five of them were polymorphic (55.56% polymorphism). Primer 17898 B produced 9 bands with fragment sizes which ranged from 1452 to 718 bp. Primer Hb2 yielded 12 bands with fragment size ranging from 1391 to 174 bp, six of them were polymorphic (50.0% polymorphism). Primer 17899A produced 11 fragments with size ranging from 1111 to 210 bp; seven of them were polymorphic (63.64%polymorphism) as shown in Table 5.

Seven unique bands in positive state were detected with molecular size of 2875, 1104 bp for the primer HB2; 479, 120 bp for the primer 17899 B; 398, 222 bp for the primer Hb2 and 1111bp for the primer 17899A. In the same context, two positive unique bands (AF01 and AF07) distinguished cultivar Misr-1. The two amplified fragments AF16 and AF34 were uniqueness to Misr-2. The molecular weight of 1111bp gave positive bands only for cultivar Shandaweel 1.

Table 4. ISSR-PCR polymorphism in the five bread wheat genotypes using five primers.

Amplicon	Mol. S (bp.)	M.1	M.2	Sh.1	L.1	L.2	MM*
Primer 844B							
AF01	2875	1	0	0	0	0	M +
AF02	2271	1	1	1	1	1	
AF03	1875	1	1	1	1	1	
AF04	1500	0	1	1	1	1	M -
AF05	1369	1	1	1	1	1	
AF06	1200	0	1	1	1	1	M -
AF07	1104	1	0	0	0	0	M +
Primer 17899B							
AF08	614	1	1	1	1	1	
AF09	536	1	1	1	1	1	
AF10	479	0	0	0	0	1	M +
AF11	414	0	1	1	1	1	M -
AF12	361	1	0	0	1	0	
AF13	298	0	1	1	1	1	M -
AF14	258	1	1	1	1	1	
AF15	176	1	1	1	1	1	
AF16	120	0	1	0	0	0	M +
Primer 17898B							
AF17	1452	1	1	1	1	1	
AF18	1339	1	1	1	1	1	
AF19	1180	1	1	1	1	1	
AF20	1069	1	1	1	1	1	
AF21	960	1	1	0	1	1	M -
AF22	900	0	0	1	0	1	
AF23	718	1	1	0	1	1	M -
Primer Hb2							
AF24	1391	0	1	1	0	0	
AF25	984	1	1	1	1	1	
AF26	867	1	0	1	1	0	
AF27	750	1	0	0	0	1	
AF28	660	1	1	1	1	1	
AF29	555	1	1	1	1	1	M -
AF30	469	1	1	1	1	1	
AF31	398	0	0	0	0	1	M +
AF32	332	0	1	1	1	1	M -
AF33	273	1	1	1	1	1	
AF34	222	0	1	0	0	0	M +
AF35	174	1	1	1	1	1	

Table 4. Continued

Amplicon	Mol. S (bp.)	M.1	M.2	Sh.1	L.1	L.2	MM *
Primer 17899A							
AF36	**1111**	**0**	**0**	**1**	**0**	**0**	**M +**
AF37	**1031**	**1**	**1**	**1**	**1**	**0**	**M -**
AF38	**900**	**1**	**1**	**0**	**1**	**1**	**M -**
AF39	**800**	**1**	**1**	**1**	**1**	**1**	
AF40	**680**	**1**	**1**	**1**	**1**	**1**	
AF41	**600**	**0**	**1**	**1**	**0**	**1**	
AF42	**549**	**1**	**0**	**1**	**1**	**1**	**M -**
AF43	**490**	**1**	**1**	**1**	**1**	**1**	
AF44	**400**	**0**	**1**	**1**	**1**	**1**	**M -**
AF45	**330**	**1**	**1**	**1**	**0**	**0**	
AF46	**210**	**1**	**1**	**1**	**1**	**1**	

***MM: Molecular marker M^+: Positive marker M^-: Negative marker**
Lane M: molecular marker, Lanes 1-5: the genotypes; Misr-1 (M.1), Misr-2 (M.2), Shandaweel-1 (Sh.1), Nesr and S8 / 17

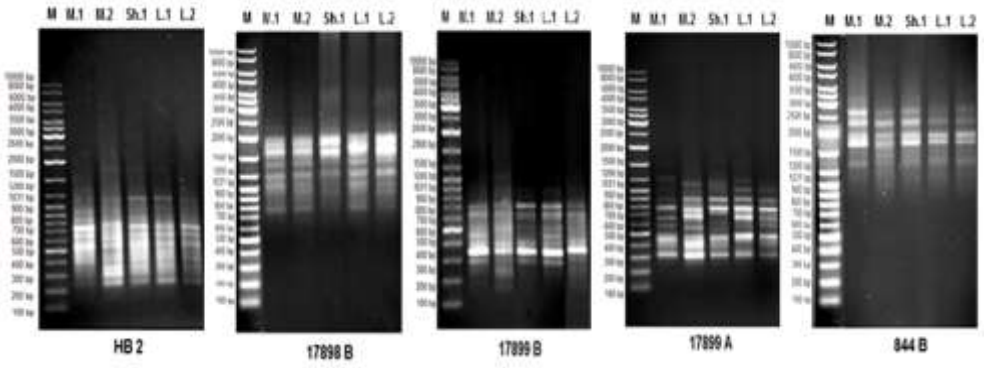

Fig. 1. ISSR fingerprints of the five bread wheat genotypes tested using HB2, 17898B, 17899B, 17899Aand 844B ISSR-PCR primers.

The two amplified fragments AF10 (47 bp) and AF31 were uniqueness to the promising line S8/17. The aforementioned bands may discriminate the salt tolerant or sensitive genotype. On the other hand, eleven negative unique bands were detected from the total of fragments. Five negative unique bands (AF04, AF06, AF11, AF32 and AF44) distinguished the cultivar Misr-1. Each of Misr -2 and the pure line S8/17 was singularity by absence of bands with molecular weight of 549 and 1031bp in case of using primer 17899A, respectively. For Shandaweel 1, three negative unique bands (AF21, AF23 and AF38) were found in the ISSR marker outputs.

Table 5. Details of the five ISSR primers for the five tested bread wheat genotypes and corresponding number of ISSR DNA markers.

Primer	TAF	PB	P%	Genotype										TSM
				G.1		G.2		G.3		G.4		G.5		
				AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
844B	7	4	57.14	5	4	5	0	5	0	5	0	5	0	4
17899B	9	5	55.56	5	2	7	1	6	0	7	0	7	1	4
17898B	7	3	42.86	6	0	6	0	5	2	6	0	7	0	2
HB2	12	6	50.00	8	1	9	1	9	0	8	0	9	1	3
17899A	11	7	63.64	8	1	9	1	10	2	8	0	8	1	5
Total	46	25	53.84	32	7	36	2	35	3	35	0	36	0	18

TAF= Total number of amplified fragments, PB = Polymorphic bands, P%= Polymorphism percentage, AF= Amplified fragments/genotype, SM= Genotype- specific marker including either the presence or absence of a given band, TSM= Total number of specific markers

The genetic diversity matrix

Based on the ISSR marker polymorphisms, diversity matrix was developed by NT-Sys computer package. The analysis depended on the number of different markers between any given pair of genotypes. The percentage of diversity between the studied genotypes (Table 6) revealed that the maximum value of diversity is 40.5% (observed between Shandweel-1 and Misr-1 genotypes), whereas the minimum value is 20.4% (observed between Nesr and S8/17 genotypes). The genetic diversity among parents was a result of differences in their pedigree (Table 1). On the other hand, the similarity based on ISSR marker between Misr-1 and Misr-2 emphasized the descent of their offspring from same origin. Desheva and Kyosev (2015) compared the genetic diversity among common winter wheat genotypes using molecular marker and origin and found a strong correlation between them.

The dendrogram illustrated in Fig. 2 classified the five bread wheat genotypes into two main clusters. One of them (Misr-1) separated as a genetically dissimilar genotype with all other genotypes. The second main cluster are divided into two sub clusters, the first involved shandweel-1 which is described as the second dissimilar genotype with all other genotypes tested. The second one contains Nesr, Misr-2 and S8/17 genotypes and the cultivars Nesr and Misr-2 was closely related.

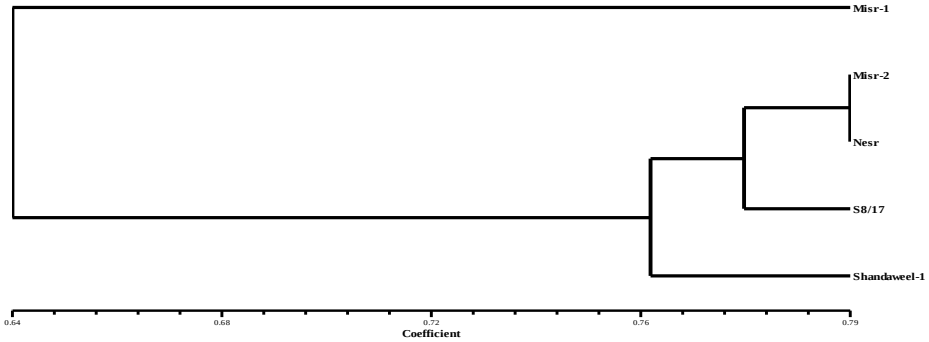

Fig. 2. Dendrogram of the five genotypes generated based on UPGM clustering method and Jacquard s coefficient using data of five ISSR markers.

Table 6. Diversity matrix among the five bread wheat genotypes based on five ISSR – PCR primers amplification analysis.

Genotype	Misr-1	Misr -2	Shandaweel-1	Nesr	S8/17
Misr-1	0				
Misr -2	0.381	0			
Shandaw	0.405	0.225	0		
Nesr	0.263	0.205	0.231	0	
S8/17	0.381	0.244	0.268	0.205	0

Table 7. Levels of genetic information generated by the five ISSR-PCR Primers on all bread wheat genotypes tested.

PIC of each primer		Index	Abbreviation	ISSR marker system
Primer	PIC			
844B	0.909	average of PIC		0.929
17899B	0.959	Number of markers	U	5.00
17898B	0.885	Number of non-polymorphic bands	n_{np}	28.00
HB2	0.950	Number of polymorphic bands	n_p	18.00
17899A	0.945	Average number of polymorphic bands/assay unit	n_p/U	3.60
-	-	Number of loci	L	46.00
-	-	Number of loci/assay unit	n_u	9.20
-	-	Total number of effective alleles	N_e	82.34
-	-	Min of PIC	$PICm$	0.885
-	-	Max of PIC	$PICx$	0.959
-	-	Fraction of polymorphic loci	B	0.612
-	-	Assay efficiency index	A_i	16.47
-	-	Effective multiples ratio	E	5.00
-	-	Marker Index	MI	4.615
-	-	Total Banding pattern	Bp	28.00
-	-	Effective number of patterns/ assay unit	P	5.60

Polymorphic information content (PIC)

The results reveal that ISSR markers generated 46 loci (Table 7). The Average number of polymorphic bands/assay unit (n_p/U) was equal to 3.6. However, the number of loci/assay unit (n_u) was 9.2 and total number of effective alleles (N_e) was 82.34. The three mention parameters seemed the most efficient by this technique. The results indicate that discrimination capacity remain one of the very important steps to evaluate the germplasm bands where numerous genotypes need to be accurately characterized and identified. The PIC per primer ranged from 0.885 for 17898B primer to 0.959 for 17899B primer with an average of 0.922.

The field trial

Mean squares of each season at each location (adequate and saline environments) revealed significant differences for all recorded traits with high mean values at Moshtohor compared with Siwa Oasis location. Results were subjected to the combined analysis of variance after performing homogeneity test as outlined by Snedecor and Cochran (1989).

Genotypic variations and locations effects

Mean squares for each and across locations of the investigated characters of bread wheat genotypes are given in Table 8.

Table 8. Mean squares of the five bread wheat genotypes tested under two diverse conditions (Mostohor and Siwa Oasis).

SOV	df	No of spikes m^{-2}	No of grains main $spike^{-1}$	1000-grain weight (g)	grain yield m^{-2}
Mostohor					
Replication	**2**	**14.47**	**1.5**	**0.17**	**7.27**
Genotypes	**4**	**10257.92****	**335.50****	**79.75****	**69424.53****
Error	**8**	**26.97**	**2.13**	**0.14**	**20.15**
Siwa Oasis					
Replication	**2**	**3.62**	**3.53**	**0.02**	**37.43**
Genotypes	**4**	**934.27****	**524.97****	**157.03****	**24776.34****
Error	**8**	**25.57**	**1.77**	**0.13**	**17.17**
Combined across location					
Location	**1**	**389196.30****	**437.85****	**248.54****	**287434.41****
Rep/L	**4**	**9.04**	**2.52**	**0.09**	**22.35**
Genotypes	**4**	**8638.69****	**830.32****	**224.88****	**83637.64****
GxL	**4**	**2553.49****	**30.16****	**11.91****	**10563.23****
Error	**16**	**26.27**	**1.95**	**0.13**	**18.66**

****: Significant at P ≤ 0.01 probability level.**

Mean squares due to the main sources of variation, i.e. genotypes (G) and location (L) as well as the interaction between genotypes and environments were significant, suggesting that all traits were markedly affected by salinity stress and the presence of a wide range of differences among genotypes concerning all investigated traits. The results confirmed

the varying genetic background of the tested genetic materials. Consequently, it could be concluded that soil and climate fluctuations significantly affected the performance of the tested bread wheat germplasm concerning most studied traits. The sensitivity of genotypes to environmental effects was reported by Afiah *et al* (2014) and AL Saadoown *et al* (2017).

Performance of yield and its attributes

The mean performances of yield and its attributes (No. of spikes m^{-2}, No. of grains main $spike^{-1}$, 1000-grain weight, and grain yield m^{-2}) for the five bread wheat genotypes under the two experimental locations (Moshtohor and Siwa Oasis) are illustrated in Table 9. Significant differences were recorded among the five genotypes for all measured traits. The stress conditions under Siwa Oasis resulted in a decrease in yield and its attributes for all genotypes.

Table 9. Mean performance of the genotypes for yield and its main attributes.

Genotype	No of spikes m^{-2}			No of grains main $spike^{-1}$		
	Moshtohor	Siwa Oasis	Combined	Moshtohor	Siwa Oasis	Combined
Misr-1	503.33A	207.25A	355.29A	48.06C	40.55D	44.31E
Misr-2	379.17C	171.08C	275.13C	55.56BC	41.75D	48.655D
Shandaweel-1	356.67D	167.00C	261.84D	53.76B	60.88B	57.32B
Nesr	376.67C	165.25C	270.96C	56.29BC	46.67C	51.48C
S8 / 17*	420.00B	186.25B	303.13B	76.25A	70.89A	73.57A
	1000-grain weight (g)			Grain yield/ m^{-2} (g)		
	Moshtohor	Siwa Oasis	Combined	Moshtohor	Siwa Oasis	Combined
Misr-1	39.99B	33.34B	36.67B	588.00B	382.67B	485.34B
Misr-2	31.94C	23.72E	27.83E	403.67E	224.00D	313.835E
Shandaweel-1	34.35C	31.32C	32.835C	439.25D	281.75C	360.50D
Nesr	33.31C	24.93D	29.12D	489.42C	383.83B	436.625C
S8 / 17*	44.18A	41.68A	42.93A	782.83A	452.08A	617.455A

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (P < 0.05)

Regarding, the number of spikes m^{-2}, the results showed that Misr-1 recorded the highest values 503.33, 207.25 and 355.29 at Moshtohor, Siwa Oasis and the combined across the two locations, respectively. The genotype S8/17, ranked second for this trait at both and across locations. Meanwhile, a lower value (261.84) was recorded by Shandaweel-1 for the combined analysis.

Concerning number of grains main $spike^{-1}$, the S8/17 genotype exceeded all genotypes and gave the highest value under adequate

conditions and salinity stressed environment and across them. On the other hand, shandaweel-1 was the second highest.

The lowest number of grains main- spike^{-1} was recorded in Misr-2 under both locations and across them.

Concerning seed index, the S8/17genotype gave the highest 1000-grain weight i.e. seed index under adequate and stress as well as combined across them, whereas Misr-2 variety had the lowest value of seed index under both and across locations.

With regard to grain yield m^{-2}, Table 9 shows that S8/17 genotype gave the highest grain yield m^{-2} with significant difference over the remaining genotypes followed by Misr-1, while Shandaweel-1 was the lowest one.

Comparing genotypes based on the tolerance indices basis

To investigate stress resistance indices for screening of wheat genotypes under Siwa Oasis habitat compared with Moshtohor habitat, grains yield m^{-2} were measured for calculating different sensitivity and tolerance indices (Table 10). A suitable index must correlate to any measured parameter under both tested conditions (Afiah *et al* 2007 and Farshadfar *et al* 2013). Grains yield m^{-2} across genotypes exhibited significant differences between stress (Siwa Oasis habitat) and normal (Moshtohor habitat) conditions. The differences varied among wheat genotypes (Table 8). The highest grain yield was given by genotype S8/17 under both normal and stress conditions followed by Misr-1 under normal environment and Nesr under the salinity stressed environment. The lowest grain yield m^{-2} under as well as salinity condition was shown by Misr-2. The variations among the genotypes obtained in the current study are in agreement with results of Fayaz and Arzani (2011) and Afiah *et al* (2014), they reported that grain yield varied considerably from adequate to stress conditions.

Since salinity is a serious problem reducing crop productivity, improvement of tolerance in crops such as wheat is a major objective for most crop breeding programs. Based on the stress tolerance index (STI) and grain yield, the S8 / 17 line was salt tolerant with the highest STI and grain yield, while the cultivar Misr-2 displayed the lowest STI and grain yield under these conditions. The genotype with high STI usually has high difference in yield under the two different conditions. In general, similar ranks for the genotypes were observed by GMP and HM parameters as well as YI, which suggests that these three parameters are equal for screening tolerant genotypes (Mevlut and Sait 2011). Nesr genotype recorded the highest stress tolerance (TOL) and SSI as well as YSI as compared with other genotypes suggesting more stress tolerance mechanism in this genotype.

Table 10. Tolerance indices of grain yield m-2 measured for 5 bread wheat genotypes cultivated under adequate and stress environments.

Genotype	Grain yield/ m^{-2}		Tolerance indices							
	Moshtohor	Siwa oasis	TOL	MP	HM	SSI	GMP	STI	YI	YSI
Misr-1	588.00	382.60	205.40	485.30	463.57	0.96	1.24	0.77	1.11	0.65
Misr-2	403.67	224.00	179.67	313.84	288.12	1.23	1.34	0.31	0.65	0.55
Shandaweel-1	439.25	281.75	157.50	360.50	343.30	0.99	1.25	0.42	0.82	0.64
Nesr	489.42	383.83	105.59	436.63	430.24	0.60	1.13	0.64	1.11	0.78
S8 / 17*	782.83	452.08	330.75	617.46	573.16	1.17	1.32	1.21	1.31	0.58

TOL: Tolerance index, MP: Mean productivity, HM: Harmonic mean, SSI: Stress susceptibility index, GMP: Geometric Mean productivity, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index.

REFERENCES

Afiah, S.A., Hanan, E. Deef and Alaa, A. Al-Shahat (2014). Inter simple sequence repeats and salt tolerance indices for assessing some bread wheat genotypes under two diverse environments. Annals Agric. Sci., Moshtohor Sp. Issue 59(2):349-364.

Afiah, S.A., K. Z. Ahmed and Kh. A. Soliman (2007). Somaclonal variation in bread wheat (*Triticum aestivum* L.). 4- RAPD Finger printing of elite genotypes under Siwa Oasis conditions. Proc.8th African Crop Sci. Conf. Proc. El-Minia, 27- 31 October 2007, Egypt 8: 2039-2045.

AL Saadoown A.W., A.A. EL-Hosary, S.A. Sedhom, M.EL.M. EL-Badawy and A.A.A. EL-Hosary (2017) Genetic analysis of diallel crosses in wheat under stress and normal irrigation treatments. Egypt. J. Plant Breed. 21 (5): 279 – 292.

AL Saadoown, A.W., A.A. EL Hosary, S. A. Sedhom., M.EL.M. ELBadawy and A.A.A. El Hosary (2018). Determination of combining ability and genetic diversity using issr markers to evaluate the genetic variability in wheat. The 7th Scientific and 1st International Conference of Agricultural Researches, Tikrit, Iraq, 10-11 April. 18: 67-80

Bidinger, F.R., V. Mahalakshmi and G.D.P. Rao (1978). Assessment of drought resistance in millet Factors effecting yields under stress. Australian Journal of Agriculture Research 38: 37-48.

Bouslama, M. and W. T. Schapaugh (1984). Stress tolerance in soybean. Part 1: Evaluation of three screening techniques for heat and drought tolerance. J. of Crop Sci. 24: 933-937.

Clarke, J.M, T.M. Towenley-Smith,T.N. McCaig and D.G. Green (1984). Growth analysis of spring wheat cultivars of varying drought resistance. Crop Sci. 24: 537-541.

Clarke, J.M., R. M. DePauw and T.F. Townley Smith (1992). Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 32: 423-428.

Dangi, R. S., M. D. Lagu, L. B. Choudhary, P. K. Ranjekar and V. S. Gupta (2004). Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers. BMC Plant Biology. 4-13.

Desheva, G. and B. Kyosev (2015). Genetic diversity assessment of common winter wheat (*Triticum aestivum* L.) genotypes. Emir. J. Food Agric. 27 (3): 283-290 .

Duncan, D.B. (1955) Multiple Range and Multiple F tests. Biometrics 11:1-42.

El-Hosary, A.A.A. and Gehan A. Nour El Deen (2015). Genetic analysis in the F_1 and F_2 wheat generations of diallel crosses. Egypt. J. Plant Breed. 19 (2): 355-373.

Farshadfar, E., M.M. Pour Siahbidi and S. M. Safavi (2013). Assessment of drought tolerance in land races of bread wheat based on resistance/ tolerance indices. Int. J. Adv. Biol. Biom. Res., 1(2):143-158.

Fayaz, N. and A. Arzani (2011). Moisture stress tolerance in reproductive growth stages in triticale (X *Triticosecale* Wittmack) cultivars under field conditions. Crop Breed. J. 1: 1-12.

Fernandez, G.C.J. (1992). Effective selection criteria for assessing plant stress tolerance. In: Kuo CG (ed) Proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Publication, Tainan, Taiwan, 257-270.

Fisher, R.A. and R. Maurer (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research 29: 897-912.

Gavuzzi, P., F. Rizza, M. Palumbo, R.G. Campaline, G.L. Ricciardi and B. Borghi (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Plant Science 77: 523-531.

Hall, A.E., (1993). Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? In: T.J. Close & E.A. Bray (Eds.), Plant Responses to Cellular Dehydration During Environmental Stress, pp. 1–10. The American Soc. Plant Pathologists, Rockville, Maryland.

Jackson, M.L. (1962). Soil Chemical Analysis. Constable and Co. LTD. London.

Kahrizi, D., M. Maniee, R. Mohammadi and K.Cheghamirza (2010). Estimation of genetic parameters related to morpho-agronomic traits of durum wheat (*Triticum turgidum* var. *durum*). Biharean Biologist 4(2): 93-97.

Kristin, A.S.; R.R. Serna, F.I. Perez, B.C. Enriquez and J.A.A. Gallegos (1997). Improving common bean perfor mance under drought stress. Crop Sci. 37: 51-60.

Lin, C.S, M.R. Binns and L.P. Lefkovitch (1986). Stability analysis: where do we stand? Crop Sci., 26: 894-900.

Madani, A. A. (2005). Soil salinity detection and monitoring using landsat data: A Case Study from Siwa Oasis, Egypt. GI Science & Remote Sensing 42(2): 171-181.

Maniee, M., D. Kahrizi and R. Mohammadi (2009). Genetic variability of some morpho-physiological traits in durum wheat (*Triticum durum* Desf.). J. Appl. Sci. 9(7): 1383-1387.

Metwaly, M.M. (2012). Ecophysiological and anatomical responses of drought stressed wheat plants (*Triticum aestivum* L.) treating with some bacterial endcphytes. Ph. D. Thesis. Faculty of Agriculture. Kafre El-Sheikh University, Egypt.

Mevlut, A. and C. Sait (2011). Evaluation of drought tolerance indices for selection of Turkish oat (*Avena sativa* L.) landraces under various environmental conditions. ZemdirbysteAgricul. 98 (2): 157-166.

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. Current Science, 80: 758-762.

Nei, N. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Piper, C.S. (1947). Soil and Plant Analysis. University of Adelaide: Adelaide.

Rohlf, F.J. (2000): NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1 Exeter Software, Setauket, N.Y.

Rosielle, A.A and J. Hamblin (1981). Theoretical aspects of selection for yield in stress and non stress environments. Crop Sci. 21: 943-946.

Singhal, N.S. and M.K. Upadhyay (1977). Genetic divergence in wheat. Cereal Res. Commun. 5: 275-286.

Snedecor, G. W. and W. G. Cochran (1989) Statistical Methods, Eighth Edition, Iowa State University Press, USA.

Taghian, A.S. and A. Abo-Elwafa(2003). Multivariate and RAPD analyses of drought tolerance in spring wheat (*Triticum aestivum* L.). Assiut J. Agric. Sci., 34: 1-24.

Tessier C., J. David, P. This, J.M. Boursiquot and A.Charrier (1999). Optimi- zation of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theor. Appl. Genet. 98:171-177.

Weising K, H. Nybom, K. Wolf and G. Kahl (2005). DNA Fingerprinting in Plants: Principles, Methods and Applications. 2nd ed. CRC Press Taylor and Francis Group, Boca Raton, USA.

انتخاب تراكيب وراثية متباعدة من قمح الخبز تحت ظروف الإجهاد الملحي بأستخدام الوسامات الجزيئية ISSR و بعض دلائل تحمل الإجهاد

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تهدف الدراسة الى تحديد و تقييم التباعد الوراثى وتحمل الاجهاد الملحى لخمسة تراكيب وراثية من القمح فى موقعين مختلفين (واحة سيوة – مشتهر) خلال موسم ٢٠١٦/ ٢٠١٧. قدر التباعد الوراثى للتراكيب الوراثية بأستعمال الواسمات الجزيئية ISSR حيث كان عدد المعلمات الناتجة من استخدام خمسة بادئات هو ٤٦ شظية و كانت نسبة الاختلافات ٥٤,٣٥%. حيث حقق البادىء 17899A اعلى نسبة اختلافات (٦٣,٦٤%). تم تحديد ٧ و ١١ شظية فريدة موجبة و سالبة على التوالى. ميزت تلك الاختلافات الاصيلة التراكيب الوراثية المتحملة للأجهاد الملحى.و بتحليل تلك الشظايا وجد ان اعلى تباعد وراثى ٤٠,٥% كان بين الصنفين شندويل-١ و مصر-١ بينما كان اقل نسبة تشابه ٢٠,٤% بين السلالتين نصر و S8/17.كان العدد الاجمالى للأليلات الفعالة ٨٢,٣٤. تراوح محتوى الانماط متعدد الأشكال لكل بادىء من ٠,٨٨٥ للبادىء 17898B إلى ٠,٩٥٩ للبادىء 17899B بمتوسط قدره ٠,٩٢٢. اختلف محصول الحبوب بالمتر المربع اختلافا معنويا بين البيئتين تحت الدراسة (واحة سيوة – مشتهر)و ايضا كان هناك اختلافات بين التراكيب الوراثية للقمح. حققت السلالة S8/17 (الناتجة من تضاعف النباتات الاحادية) اعلى محصول للحبوب/م٢ و تلاها الصنف مصر-١. حققت السلالة S8/17 اعلى دليل التحمل للإجهاد (STI) و محصول الحبوب/م٢ بينما حقق الصنف مصر-٢ اقل القيم. و لوحظت نفس النتائج عند استخدام دليل تحمل الاجهاد ومتوسط الانتاجية الهندسى (GMP) و ايضا متوسط التناسق (HM) و كذلك عند استخدام مؤشر دليل المحصول (YI). اظهرت السلالة نصر اعلى مؤشر تحمل الاجهاد (TOL) ودليل الحساسية للإجهاد (SSI) و كذلك دليل ثبات المحصول (YSI). مما يشير الى وجود ميكانيكيات تحمل الاجهاد الملحى فى تلك التراكيب الوراثية.

المجلة المصرية لتربية النبات ٢٣ (١) : ٧٧- ٩٢ (٢٠١٩)