Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2):963-978. **OPEN ACCESS**

Validation of molecular markers linked with salinity tolerance in wheat (*Triticum aestivum* L.) grown on saline soil.

ELshafei A.A.[1*], Afiah S.A.[2], Amer M.A[3] and Magda A.M. El-enany[1]

[1]Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Centre (NRC), El-Dokki, Cairo, **Egypt.**
[2]Genetic Resources Dept., Desert Research Center, El-Matareya, Cairo, **Egypt.**
[3]Genetic Resources Research Dept. in Field crops research institute, ARC, Giza, **Egypt**

***Correspondence:** elshafei_2000@yahoo.com **Accepted:** 31Jan.2019 **Published online:** 12 Apr. 2019

Performance of nine lines with two local bred wheat genotypes check, were evaluated during 2016-17 at two locations, Siwa Oasis and Ashmon in Menofeya, Governorate. The eleven bread wheat genotypes were examined for their grain yield under salinity stress (YS) and non-stress (Yb). Owing to the differences in the salinity levels at the two locations. In both areas, grain yield was documented at maturity. According to grain yield data, stress susceptibility index (SSI), stress tolerance index (STI), tolerance index (TOL), harmonic mean (HM), modified stress tolerance index (k1STI & k2STI), mean productivity (MP), geometric mean productivity (GMP), yield stability index (YSI) and stress susceptibility percentage index (SSPI) were calculated. The highest STI, TOL, HM, MSTIk1, MSTIk2, MP, GMP and SSPI values were calculated from genotype L2 followed by L4 and L6, proving that those genotypes had the highest salt sensitivity and grain yield reduction under salt stress condition(YS), on the other hand, the lowest ones were found in L3, G171and L9. The association analysis of the 24 SSR marker data for YS, Yb, and the ten previous indices showed high determination coefficients (R2) between molecular markers and all of the indices. The molecular markers wmc504, wmc11, xgwm350 and xgwm205 were the best SSR markers for explaining the indices variability in wheat genotypes.

Keywords: Salt Tolerance Indices, SSR markers, Wheat breeding, Determination coefficients.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the ultimate significant grain crop in the universe and covers the earth's surface more than any other food crop. Wheat is the main staple food in Egypt, and it inhabits a great level in the cropping configuration of the country. Therefore, a large deal of research work is conducted in the field of wheat breeding through genetic variability. However, any increase in population and climatic changes in the country necessitate further breakthroughs in wheat breeding. Reaching a development in the heritable traits and estimating genetic parameters is of paramount significance in any breeding program. Increasing wheat production to decrease the gap between production and consumption is the main issue of wheat breeders. Despite many efforts of wheat breeders, yield losses due to abiotic stresses (water stress, salinity or high temperature) and biotic stresses (diseases, insects, etc.) are still considered the main constraints to obtain a high grain yield.

Selecting salinity stress tolerant genotypes based on the physiological traits only, might affect productivity (Zhu et al, 2016). Moreover, salinity stress can significantly reduce grain yield because plants assign a considerable amount of its

metabolic process and energy to adaptation, maintain growth and respond to stress, which often resulted in yield reduction (Stavridou et al., 2017). Grain yield is the ultimate substantial parameter that might simply define breeders' prosperity. Thus, most breeders focus on predicting or measuring the ability of genotypes to maintain yield under salinity stress conditions relative to controlled conditions as a measure of salinity stress tolerance (Negrão et al., 2017). Several stress tolerance indices (STI) have been developed to identify and to screen stress-tolerant genotypes (Mitra, 2001), which measure stress intensity based on yield decrease under stress conditions proportional to controlled conditions. Stress susceptibility index (SSI) (Fischer and Maurer, 1978) was used to measure the relative yield reduction across different environmental conditions as an indicator of stress tolerance (Thiry et al., 2016). The use of Stress tolerance index (STI) was helpful to recognize landraces that leads great yield under both stressed and natural growth circumstances (Fernandez, 1993). Several other tolerance indexes were developed and used to select tolerant genotypes grown under abiotic stress conditions (Bouslama and Schapaugh, 1984; Negrão et al., 2017; Singh et al., 2015; Zarei et al., 2007). Even though, stress tolerance indexes found to be effected in the selection for abiotic stress tolerant genotypes, it is often difficult to directly select for quantitative traits such as salinity stress tolerance under the field conditions because of the difference among genotypes in number of days to flowering, the effect of the uncontrolled environmental factors such as perception and other climate factors which might affect the selection efficiency (Ashraf and Foolad, 2013).

The evaluation of assumed results of different Physiological Indices for Salinity Stress Tolerance in Wheat studied by (Katerji et al., 2000; Singh, 2004; Ashraf et al., 2006; Shahzad et al., 2012; Ahmad et al., 2013 and Barakat et al., 2016) and reported that, regarding the use of water stress day index in the classification of salt tolerance, maize, sunflower and potato were classified together in the same group as sugar beet and durum wheat (Katerji et al., 2000). plant height and dry matter stress tolerance indices (PHSTI, DMSTI), germination stress tolerance index (GSTI), relative saturation deficits (RS cell membrane stability (% injury) and D) of the germinating seeds were valued in all compliances under 0 or 1.5 % Na cl level.

As an observation by (RAPD) and (SSR) analyses, genetic relationships and variations among a variety of wheat genotypes had different answers to salt strain (Moghaieb et al., 2011). Nine Egyptian wheat cultivars, namely, Sahel-1, Giza-168, Giza-160, Gemmiza-7, Gemmiza-10, Gemmiza-9, Sids-1, Sohag, and Beni-Suef were subjected to salt stress for two weeks. Out of the 118 RAPD markers detected, 82 (69.5%) were polymorphic, and out of the 59 SSR alleles, 42(71%) were polymorphic. Eighteen RAPD and thirteen SSR markers were genotype-specific. The cultivar Beni-Suef was distinguished by seven markers, Sohag by six markers, and Gemmiza 10 by two markers. These markers were confirmed as genetic markers linked with salt tolerance in three wheat genotypes and were applied in marker-assisted selection breeding programs. The genotypic variation among the cultivated durum wheat cultivars has been assessed using gSSRs to determine the genotypic difference of 64 durum lines, lines, and the varieties (Eujayl et al. 2002). The goals of the present study were: (1) comparative evaluation of genetic diversity between agronomic traits and molecular markers by SSR under salinity stress in eleven wheat lines, and (2) effectiveness of molecular markers linked with salt tolerance in eleven wheat lines.

MATERIALS AND METHODS

Eleven wheat genotypes were used in current study, i.e., two recently released cultivars ("Giza171" and "Sids12") and nine elite lines potentially tolerant to salinity stress, i.e., "L_1" to "L_9", information about seed sources, selection history and pedigrees are presented in the supporting information Table 2. The studied materials were planted in two locations; the Experimental Farm of the Desert Research Center located in Siwa Oasis (29°12″N, 26°3″ E) and a Grower farm located in Ashmon (30° 18′16″ N, 31° 2′ 5″ E) during the last week of November during 2016/2017 growing season. Siwa is an urban oasis in Egypt between the Qattara Depression and the Great Sand Sea in the Western Desert, nearly 50 km east of the Libyan border, and 560 km from Cairo (Northwest of the western desert). While, Ashmon located in the middle delta, 42 km from Cairo (Northern Egypt). Soil samples (0–30 cm depth) were collected during November directly before planting and analyzed according to (Klute et al., 1994). The main soil physical and chemical properties are presented in Table 1a and 1b (supporting information).For each location, three replicates in a randomized complete block design were used, in which the

eleven genotypes were assign randomly to each plot within each replicate and location. The experimental units (plots) were 3.5 meters long and 15 rows wide with 20 cm between rows. Standard agronomic practices, for each location, including recommended fertilization and irrigation schedules were followed.

Phenotypic Measurements and Tolerance Indices:

All plants in each plot were cut at 5 cm above soil service and left to dry in the middle of the plots. After three days, plants from each plot were threshed separately using locally made single plot thresher, in which seeds and straw were collected, paged, numbered then dried and weighted. Finally, grain yield measurements were used to estimate the following stress tolerance indices:

Stress Susceptibility Index (SSI):

$SSI = (\left[1 - \left(\frac{Yp}{Ys}\right)\right] / \left[1 - (\frac{\bar{Y}p}{\bar{Y}s})\right])$ (Fischer and Maurer, 1978)

Stress Tolerance Index (STI):

$STI = \frac{(Ys)(Yp)}{(\bar{Y}p)^2}$(Fernandez, 1992)

Tolerance Index (TOL):

$TOL = Yp - Ys$(Rosielle and Hamblin, 1981)

Harmonic Mean (HM):

$HM = 2(Yp \times Ys)]/(Yp + Ys)$(Schneider et al., 1997)

Modified stress tolerance index; K1 MSTI, = *Y2p/Ȳ2p* and K2 = *Y2s/Ȳ2s* (Farshadfar and Sutka, 2002), where ki is the correction coefficient.

Mean productivity; MP = (*Ys* + *Yp*)/2 (Rosielle and Hamblin, 1981) the genotypes with high MP values are more desirable.

Geometric mean productivity; GMP = $\sqrt{(Ys)}.(YP)$ (Fernandez, 1992); the genotypes with high GMP values, will be more desirable.

Stress susceptibility percentage index; SSPI= [*Yp*−*Ys*/2(*Ȳp*)] × 100. (Moosavi et al., 2008).

Yield stability index; YSI =*Ys/Yp* (Bouslama and Schapaugh, 1984); the genotypes with high YSI values are regarded as stable genotypes under stress and non-stress conditions.

Where, *Ys* and *Yp* are the average grain yield for each genotype within stressed and non-stressed environmental conditions, respectively. While, *Ȳs* and *Ȳp* are the grain yield means across all genotypes within stressed and non-stressed environmental conditions, respectively.

DNA Extraction:

DNA was extracted from all wheat genotypes using the Wizard Genomic DNA purification Kit (Promega Corporation Biotechnology, Madison, Wisconsin, USA). Then the extracted DNA was treated with RNase and reserved in a −20 °C refrigerator. The quality of DNA was assessed using 0.8% agarose gel and Epoch Multi-Volume Spectrophotometer (Biotek, Winooski, VT, USA). Before conducting the SSR analysis, DNA was diluted to 25 ng/µL concentration.

Table 1a: Soil physical analysis of the two experimental sites at Ashmon - Menofya, and Siwa Oasis during 2016/2017growing season.

Location	Physical Analysis (%)				Texture
	Course sand	Fine sand	Silt	Clay	
Ashmon	6.35	12.8	16.4	64.5	Clay
Siwa Oasis	9.8	12.4	60.82	16.98	Sandy loam

Table 1b: Chemical analysis of soil saturation extract and irrigation water during 2016/2017 growing seasons.

Location	OM (%)	$CaCO_3$ (%)	PH	EC (dSm^{-1})	Anions (meq/L)			Cations (meq/L)			
					Cl^-	HCO_3^-	SO_4^{2-}	Na^+	K^+	Ca^{2+}	Mg^{2+}
Soil analysis (0-30cm)											
Ashmon	1.7	1.6	7.5	1.8	9.8	1.15	7.1	8.7	0.35	5.7	3.2
Siwa Oasis	0.53	17.5	7.9	12.3	83.6	2.3	36.2	68.9	1.60	34.5	17.4
Irrigation water analysis											
Ashmon	-	-	7.6	1.35	1.87	3.56	6.92	12.3	0.32	1.15	1.18
Siwa Oasis	-	-	7.3	3.96	18.6	10.8	7.48	22.1	0.45	8.3	8.7

Table 2: Name, origin, and pedigree and/or selection history of eleven bread wheat genotypes tested.

Name	Origin	Pedigree and/or selection history
L1 (I-3)	ICARDA†	CM59456-3AP-1AP-2AP-1AP-0AP
L2 (L-606††)	Egypt	RCB-61// (Atlas 66 / Nap Hall) /2* RCB-61Su606-13Su-2Su-5Su-0Su-18Su
L3 (A 305)	ACSAD‡	Bb / Nar 67//Kal 1227 A / Bb /3/ JBE4-Toluca 73
L4 (S8/17††)	Egypt	R8 tissue culture-regenerated double-haploid plant
L5 (Gomam)	CIMMYT§	SWM 11619-2 AP-4 AP-1 AP-2 AP-0AP
L6 (I-6)	ICARDA†	Dove "S" / Buc "S" CM58808-6AP-2AP-1AP-1AP-0AP
L7 (Nesser)	CIMMYT§/ICARDA†	ICW85-0024-06AP-300AP-300L-1AP-0AP
L8 (L-263††)	Egypt	Sids 1 // CM 33204 7Su-26SW-3SW-1SW-0SW
L9 (Siwa5)	Egypt	Newly bred line selected under Siwa Oasis conditions
Sids12	Egypt	BUS//7C//ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL// CMH74A.630/4*SX.- SD720096-4SD-1SD-1SD-0SD
GIZA 171	Egypt	SAKHA 93/GEMMEIZA 9 - S.6-1GZ-4GZ-1GZ-2GZ-0S

†ICARDA: International Center for Agricultural Research in the Dry Areas.
‡ACSAD: The Arab Center for the Studies for Arid zones and Dry lands.
§CIMMYT: Centro International de Mejoramiento de Maize Y Trigo (Mexico) = International Maize and Wheat Improvement Center.
†† Newly bred lines released through the Desert Research Center wheat breeding program.

Thirty four different specific SSR markers linked to salinity tolerance in wheat were used (Table 4). These microsatellite primers were developed by several investigators (Lindsay et al., 2004; Byrt et al., 2007; Shahzad, 2007; Byrt, 2008; Huang et al., 2008; Lindsay et al., 2008; Ahmad, 2011; James et al., 2011)). A polymerase chain reaction (PCR) mixture consisted of 20 to 50 ng of genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM each dNTP, 0.5 µM each of forward and reverse primers, and 1 U Taq polymerase in a volume of 0.025 cm^3. The PCR program for the SSR analyses included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50, 52, 55, 60 and 61 °C (depending on the individual SSR primers) for 1 min, and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 10 min. The amplified PCR products were applied to 3% (m/v) agarose gel containing 0.1µg cm^{-3} ethidium in TBE buffer. After electrophoresis a photograph of the gel was taken by on a UV trans-illuminator. The EST-SSR and SSR data were scored on the bases of presence (1) or absence (0) of a given marker, after excluding irreproducible bands.

Statistical Analysis:

Phenotypic Data:

Analysis of variance was carried out using SAS 9.2 (SAS v9.2; SAS Institute Inc., Cary, NC, USA), by fitting the following linear model (Federer et al., 2007):

$Y_{ijm}=\mu+ E_i + EB_{(i)j} + G_m + EG_{im} + \varepsilon_{ijm}$

Where Y_{ijm} is the response measured on the $_{ijm}$ plot, µ is the overall mean, E_i is the effect of i^{th} Environment (two locations), EB $_{(i)j}$is j^{th} block nested within i^{th} environment, G_m is the effect of m^{th} genotype, EG_{im} is the interaction effect among i^{th} environment, and G^{th} genotype, and ε_{ijm} is the experimental error.

Means were compared using the new *LSD* test (at *P-value* < *0.05*), according to Gomez and Gomez (1984). Homogeneity of the variance across environments was tested following Bartlett's Test (Steel and Torrie, 1980).

Molecular Marker Data and Genetic Variability:

A similarity matrix (SM) was estimated according to Nei and Li (1979) using molecular marker data as following:

$SM = 2N_{ij}/(N_i + N_j)$ (Nei and Li, 1979)

where, N_{ij} is the number of bands present in both i^{th} and j^{th} genotypes, N_i is the number of bands present in i^{th} genotype, and N_j is the number of bands present in the j^{th} genotype.

The similarity matrix was then subjected to the unweighted pair group method with arithmetic average (UPGMA) clustering algorithm. Principal coordinate analysis (PCoA) was used as an alternative to hierarchical clustering in that the similarity matrix was used to obtain the coordinates. These coordinates were then used to create scatter plots that represent the relationships among genotypes. Both UPGMA and PCoA were conducted using PAST software version 1.62 (Hammer et al., 2001). Furthermore, to evaluate the reliability of the generated dendrogram, 1000 simulations were performed using the PAUP software version 4.0b10 (Swofford, 2001). Polymorphic information content (PIC) was calculated as following: $PIC = 1 - \sum_{i=1}^{n} p_i^2$(Smith et al., 1997)

Where, p_i is the frequency of the i^{th} allele across genotypes. The discrimination power was calculated by dividing the number of polymorphic

alleles amplified for each primer by the total number of polymorphic alleles obtained (Khierallah et al. 2011).

To identify the informative markers, and study the correlation between genetic diversity and Stress Tolerance Index, the association analysis between molecular data of the SSR markers was carried out using Map Manager QTX software (Manly et al., 2001).

RESULTS AND DISCUSSION

Comparison of genotypes based on salinity tolerance indices:

Regarding the data, the average yield of bread wheat genotypes under stress environment was from1.94 to 4.32 gm plant-1, but was from 2.23 to 5.23 gm plant-1 in genotypes under non-stressed environment. In (Table 3), L2, L4 and L6 genotypes were of highest results in grain yield at salt stressed and non-stressed conditions, and of high significant differences for all the salt tolerance indices. Also, the values of salt tolerance screening methods and the genotypes adaptations to various locations are presented in table 3. The largest STI, TOL, HM, MSTIk1, MSTIk2, MP, GMP and SSPI, values were recoded for L2, followed by L4 and L6, proving that the genotypes had greatest grain yield (YS) reduction under salt stress condition and highest salt sensitivity, while, the lowest STI, TOL, HM, MSTIk1, MSTIk2, MP, GMP and SSPI, values were found in L3, G171 and L9 respectively, proving that, those genotypes had a lowest grain yield reduction in stress condition. According to YSI, the genotype G171, followed Sd12, L1, L3 and L9 had the highest values, indicating that these genotypes had a lower grain yield (YS) reduction under salt stress condition and higher salt sensitivity while, genotype L5, followed by L6, L4 and L2 were of the lowest values. For SSI values the genotype L5, followed L4, L6 and L2 were of highest values while, genotype G171, followed by Sd12, L1 and L3 had the lowest values. Based on the ranking of STI, TOL, HM, MSTIk1, MSTIk2, MP and GMP, genotypes L2, L4 and L6 had the highest values, (1, 2 and 3 respectively,) the similar ranks of the genotypes for STI, TOL, HM, MSTIk1, MSTIk2, MP and GMP parameters, whereas the rest of genotypes had the lowest values. Similar ranks of the genotypes for STI, TOL, HM, MSTIk1, MSTIk2, MP and GMP parameters assume that those three indices are equivalent for choosing genotypes. Several studies (e.g., Zeynali et al., 2004; Talebi et al., 2009; Sanjari and Yazdansepas, 2008; Nouri et al., 2011 and Mohammad et al., 2010) indicated that the most suitable parameters for screening stress-tolerance in plants were their tolerance indices and high-yielding potentiality.

Correlation Analysis:

In comparison to the non-stressed environment, the mean yield of the eleven genotypes in the stressed environment was reduced by 31(%), so the genotypes experienced a moderate/tolerant salt stress.

The correlation coefficient between YP, Ys and quantitative indices of stress tolerance were calculated (Table 6). The yield under salt-stressed conditions (Ys) had a very highest association with the yield under non-stressed conditions (YP), suggesting that the crop could be significantly improved under saline condition. For example, the genotypes L2, L4 and L6, produced the highest yield under non-stressed and stress conditions. There were significant and correlations among YP, YS and all salt stress indices, (SSI, STI, TOL, HM, MSTIk1, MSTIki, MP, GMP and SSPI). The correlation values among YS, and (SSI, STI, TOL, HM, MSTIk1, MSTIki, MP, GMP and SSPI), indices were positively correlated and significantly ($r = 0.707$, $r = 0.968$, $r = 0.847$, $r = 0.997$, $r = 0.981$, $r = 0.864$, $r = 0.996$, $r = 0.988$ and $r = 0.832$, respectively) and was negatively correlated with YSI ($r = -0.513$) ($P \leq 0.01$) under salinity stress (Table 6). Assessments of multiple tolerance indices of salinity stress in bread wheat (*Triticum aestivum* L.) were reported by (Singh et al., 2015, Zhu et al., 2016 and Thriy et al., 2016). The Ys and YP viewed great significance and positive associations with GMP, MP and STI among the studied indices. As a result, these indices were better forecasters of Ys and YP than TOL, SSI and YSI, in bread wheat (Singh et al., 2015). Generally, almost all of the bread wheats presented better Na+ exclusion that was related with higher relative yield (Zhu et al., 2015). This stress indices SSI, STI, MP, GMP, and TOI, could help breeders and researchers by defining clear and strong criteria to identify genotypes with high resilience and high productivity and provide a clear visualization of contrasts in terms of grain yield production under stress (Thriy et al., 2016). Hosseini et al., (2012) found that five salt tolerance indices comprising: (TOL), (MP), (GMP), (STI) and (SSI), were used. As a conclusion, the potential of these genotypes tolerating salt stress was found to high MP, STI and low SSI for both shoot length and root dry weight.

Table 3: Salt tolerance indices of eleven bread wheat genotypes based on grain yield per plant for stress and adequate conditions.

Gen.	YS(gm/p)	YP(gm/p)	SSI	STI	TOL	HM	MSTI K1	MSTI K2	MP	GMP	SSPI	YSI
(L1)	2.77 (5)	3.03(6)	0.09(9)	0.751(9)	0.26 (8)	2.894 (5)	0.822 (6)	0.931(9)	2.9(6)	4.20(5)	3.89(9)	0.914(3)
(L2)	4.32 (1)	5.23 (1)	0.17(4)	3.146(1)	0.91 (1)	4.732 (1)	2.448 (1)	3.623(1)	4.78(1)	11.3(1)	19.09(1)	0.826(8)
(L3)	1.94(10)	2.2 (11)	0.12(8)	0.594(11)	0.26 (9)	2.062 (11)	0.433 (11)	0.457(11)	2.07(11)	2.13(11)	5.45(8)	0.882(4)
(L4)	3.88 (2)	4.74(2)	0.18(2)	2.561(2)	0.86 (2)	4.267 (2)	2.011 (2)	1.827(2)	4.31(2)	9.20(2)	18.04(2)	0.819(9)
(L5)	2.6 (7)	3.22(5)	0.19(1)	1.166(5)	0.62 (4)	2.877 (6)	0.928 (5)	1.312(5)	2.91(5)	4.19(6)	13.01(4)	0.807(11)
(L6)	3.52(3)	4.3(3)	0.18(3)	2.107(3)	0.78(3)	3.871(3)	1.655 (3)	1.503(3)	3.91(3)	7.57(3)	16.36(3)	0.819(10)
(L7)	2.48(9)	2.86(7)	0.13(7)	0.988(7)	0.38(6)	2.656(8)	0.732 (7)	1.194(7)	2.67(8)	3.55(8)	7.97(6)	0.867(6)
(L8)	2.96(4)	3.47(4)	0.15(5)	1.430(4)	0.51(5)	3.195(4)	1.078 (4)	1.063(8)	3.22(4)	5.14(4)	10.70(5)	0.853(7)
(L9)	1.94(11)	2.23(10)	0.13(6)	0.602(10)	0.29(7)	2.075(10)	0.445 (10)	0.731(10)	2.09(10)	2.16(10)	6.08(7)	0.870(5)
Sids12	2.56(6)	2.7(8)	0.06(10)	1.014(6)	0.18(10)	2.697(7)	0.697 (8)	1.323(4)	2.7(7)	3.64(7)	3.78(10)	0.935(2)
Giza171	2.61(8)	2.79(9)	0.05(11)	0.962(8)	0.14(11)	2.628(9)	0.652 (9)	1.272(6)	2.63(9)	3.46(9)	2.94(11)	0.948(1)
Mean	2.87	3.34	0.13	1.39	0.47	3.09	1.08	1.39	3.11	5.14	9.76	0.87

SSI: Stress Susceptibility Index, SSTI: Stress Tolerance Index, TOL: Tolerance Index, HM: Harmonic Mean; MSTI: modified stress tolerance index; MP: mean productivity; GMP: geometric mean productivity; SSPI: stress susceptibility p

Huge differences among genotypes were noticed for grain yield. Grain yield under irrigated environment (YP) was definitely and significantly associated with MP, HM, GMP, STI and k1STI.
In the same way, positive and significant association has also been noticed between grain yields under stress condition (Ys) and MP, HM, GMP, STI, YI and k2STI. According to Fernandez model; genotypes No. 2, 4, 6, 7, 9 and 13 have identical dominance under both conditions (stress and irrigated). Genotypes No. 1, 11, 15, 16, 17, 18 and 19 were chosen for irrigated conditions. Genotypes No. 3 and 5 were suitable for stress conditions (Anwar et al., 2011).The choice of stress tolerance indices for the selection of tolerant inbred lines and their hybrids under normal and water-stress conditions examined by Papathanassiou (et al., 2015).
They concluded that, the inbred lines and the hybrids were examined using grain yield and for each genotype, B value and nine other stress indices based on their yield under typical and water stress conditions were calculated, including (SSI, MRP, TOL, MP, relative efficiency index (REI), STI, GMP, YI, HM. It was found a strong and positive correlation ($P<0.001$) of B values with all indices, except SSI, for all locations. As a result, the B value matches the ability of the other stress indices to identify drought sensitive and tolerant genotypes.

SSR analysis:

Genetic diversity of molecular markers:
Out of the 34 different SSR primer pairs used in this study, only 24 generated polymorphisms among the eleven wheat genotypes. A cluster analysis was performed on the basis of similarity coefficients generated from the SSR data. The cluster analysis using the SSR data, grouped the eleven wheat genotypes into two main clusters with similarity coefficients ranging from 0.086 to 0.88. The highest genetic similarity was observed between genotypes L1 and L5 (0.88) and the lowest one (0.086) was between L3 (salt-sensitive genotype) and L6 (salt-tolerant genotype) (Table 4 and Fig. 1). The first cluster supported by a bootstrap value of 75(%) contained seven genotypes, (L7, L9, Sd12, L3, L5, L1 and L8), which were salt-sensitive genotypes, and consisted of four subgroups. The first subgroup supported by a bootstrap value of 45(%) included one genotype L7. The second subgroup supported by a bootstrap value of 58(%) included three wheat genotypes (L9, Sd12 and G171). The third subgroup supported by a bootstrap value of 65(%) included three wheat genotypes (L3, L5 and L1). The fourth subgroup supported by a bootstrap value of 75(%) included one wheat genotype (L8) (Fig. 1). The second cluster supported by a bootstrap value of 81(%) contained three wheat genotypes (L4, L6 and L2) which, were salt-tolerant (Fig. 1). These results suggested that cluster analysis could be used to isolate the salt-tolerant genotypes apart from the sensitive genotypes.
The findings of Prasad et al. (2000) reported that, the cluster prepared on the foundation of similarity matrix, using the UPGMA algorithm, delineated the above genotypes into two main gropes (I and II), each with two sub-clusters (Ia, Ib and IIa, IIb) in 55 wheat genotypes with twenty SSR primers. Plaschke et al. (1995) reported that the cluster analysis using 23 (wmc) SSR primers in forty wheat cultivars and lines grouped data into one main cluster, which consisted of three subgroups related by pedigrees. Al-Doss et al. (2011) reported that the UPGMA dendrogram separated the six durum wheat genotypes into three clusters with nineteen (SRAP) primers. Kumar et al. (2016) supported that, the cluster analysis in general grouped fifty four genotypes into four groups represented as A, B, C, and D. Among these four clusters, cluster D included the maximum number of genotypes (43), which were further divided into seven sub-clusters using 39 SSR markers. In general, the diversity measurements were higher in the genotypes, suggesting that such a high level of genetic similarity might be used for selecting materials in the breeding programs; wherein the genotypes with high genetic distance could be used.

Levels of genetic information generated by SSR primers:

Twenty-two SSR primers were used to investigate their (DP) by calculating the (PIC) of their loci. A total of 33 alleles, were amplified among eleven wheat genotypes, using 24 SSR primers. The number of amplified alleles per primer varied from one allele, as for barc273 primer, to two alleles, as for Nax2 primer, with an average value of 1.2768 alleles (Table 4). The sizes of the amplified alleles ranged from 90 to 620bp. The standard of polymorphism among the eleven lines was estimated by calculating the PIC values for each of the twenty four SSR loci. The PIC values varied greatly for all SSR loci tested. Sixteen SSR primers detected a single allele, and their PIC values were zero.

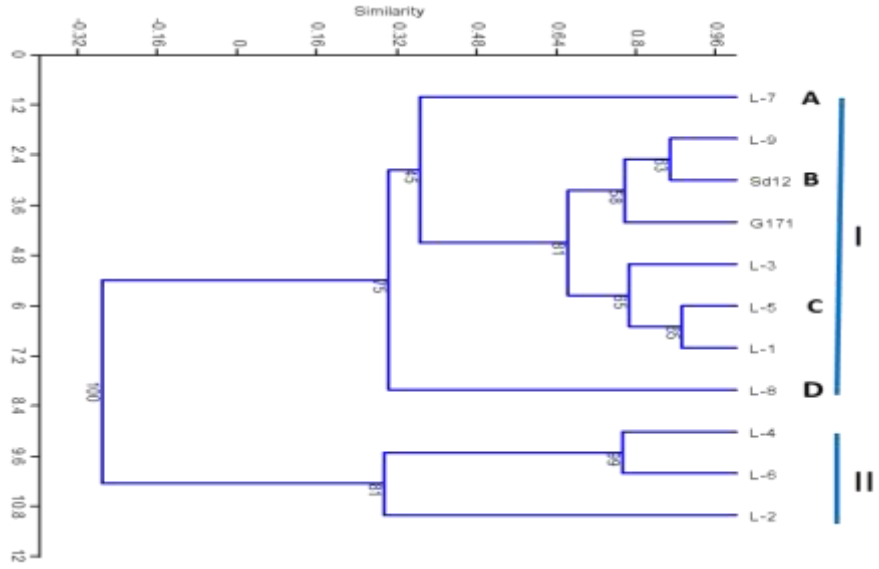

Figure 1: Un-weighted pair group method with arithmetic average (UPGMA) dendrogram for eleven wheat genotypes based on the allelic data of 24 SSRs.

Table 4: Characterization of 24 genomic-SSR makers in wheat.

Primers	Reference	Size (bp)	T_A	Polymorphic	PIC	Dp
barc12	(Shahzad 2012)	100-90	51	2	0.18	0.061
barc124	(Shahzad 2007; Ahmad 2011)	260-230	52	2	0.345679	0.061
barc182	(Shahzad 2012)	620-100	58	2	0.554017	0.061
barc273	(Munir et al., 2013)	230	52	1	0	0.030
cfd60	(Munir et al., 2013)	220	60	1	0	0.030
cfd66	(Shahzad 2012: Munir et al 2013)	270	60	1	0	0.030
cfd18	(Ahmad 2011)	95	60	1	0	0.030
Nax2	(Huang et al., 2008; James et al., 2011)	280	58	3	0.576	0.090
xgwm148	(Munir et al., 2013)	190-105	60	2	0.444444	0.061
xgwm205	(Ahmad 2011)	150	60	1	0	0.030
xgwm291	(Lindsay et al., 2004, Byrt et al., 2007; Byrt 2008, Shahzad 2012)	190	60	1	0	0.030
xgwm299	Genc et al., 2010	95	55	1	0	0.030
xgwm335	(Shahzad 2012)	170	55	1	0	0.030
xgwm340	Mardi et al., 2011	150	60	1	0	0.030
xgwm350	(Shahzad 2012)	130	55	1	0	0.030
wmc11	(Ahmad 2011)	150	61	1	0	0.030
wmc17	(Ahmad 2011)	190	51	1	0	0.030
wmc18	(Ahmad 2011)	140	61	1	0	0.030
wmc96	(Ahmad 2011)	230	61	1	0	0.030
wmc154	(Ahmad 2011)	240	61	1	0	0.030
wmc432	(Ahmad 2011)	100	51	2	0.44451	0.061
wmc503	(Ahmad 2011)	300-160	51	2	0.444444	0.061
wmc504	(Ahmad 2011)	220	51	1	0	0.030
wmc661	(Ahmad 2011)	250-110	61	2	0.35503	0.061
Total	–	–		33	2.7681	1.00
Mean	–	–		1.25	0.1393	0.042

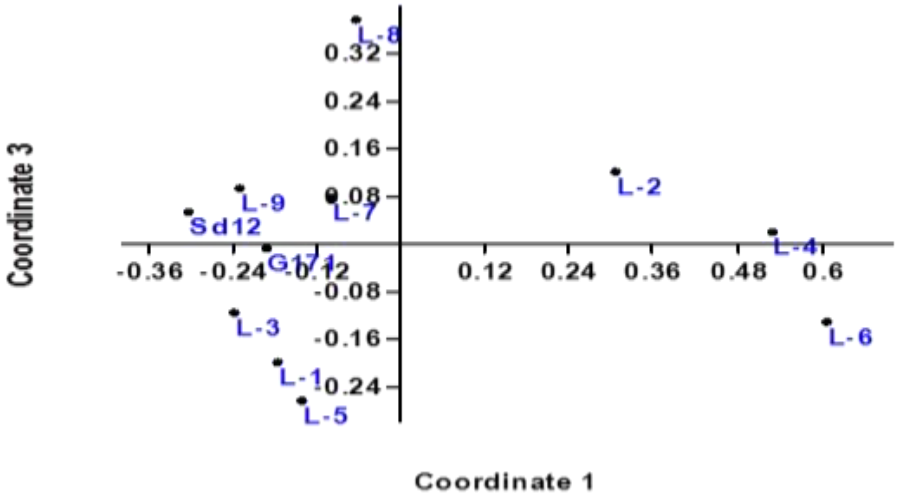

Figure 2: Principal coordinate analysis (PCoA) of the eleven wheat genotyped with 24 polymorphic SSRs.

The PIC values of the remaining seven primers ranged from 0.18 (barc12) to 0.576 (Nax2) (Table5).

The PIC values were positively correlated (r = 0.95) with the number of amplified alleles per primer. Prasad et al., (2000) reported one to thirteen alleles per locus in 55 wheat genotypes with twenty SSR markers, with a PIC value ranging from 0.21 to 0.90 and an average of 0.68. The coefficient of similarity matrix ranged from 0.05 to 0.88, with an average of 0.23. Mardi et al., (2011) reported two to ten alleles per locus in 122 durum wheat genotypes, with nineteen SSR markers. Al-Murish et al., (2013) reported an average of 2.31 amplified alleles per primer among seventeen coffee accessions using sixteen SSR primers and an average PIC value of 0.43.

The observed DP calculated for each primer ranged from 0.033 to 0.067, with an average of 0.042(Table 4).The highest DP (0.067) was observed in seven primers, such as primer barc124. The lowest DP (0.022%) was observed in 22 SSR primers, such as primer cfd60 (Table 4). Khierallah et al., (2011) reported a DP ranges from 0.31 to 0.06 among eleven date palms, using six (AFLP) combination primers. Ammar et al., (2015) reported a DP ranges from 0.29 to 0.05, using six SRAP combination primers, and 0.13 to 0.42, using the four AFLP combination primers among forty faba beans.

The principal coordinate analysis (PCoA) plot for the first Three coordinates, explaining 84.659(%) of variance among genotypes, is shown in Fig. 2. Genotypes were divided into two main clusters presenting salt tolerant genotypes and salt sensitive genotypes. PCoA display identical results as for dendrogram, thus supported, the results of UPGMA clustering. Supported the results by Hassanein and AL-Soqear (2018), Genotypes were divided into two main clusters presenting Mo genotypes and Mp genotypes. The (PCoA) plot for the first two coordinates, explaining 51.68% of variance among genotypes.

Table .5 associations of SSR markers with YS, YP and ten salt tolerance indices of wheat genotypes.

Characteristic	Informative Marker	Coefficient of Determination R^2 (%)	Significance level	Characteristic	Informative Marker	Coefficient of Determination R^2 (%)	Significance level
YS	Wmc504	82	0.00001	MP	Wmc504	84	0.00001
	Wmc11	82	0.00001		Wmc11	84	0.00001
	Xgwm350	82	0.00001		Xgwm350	84	0.00001
	Xgwm205	82	0.00001		Xgwm205	40	0.01770
	Nax2	46	0.00885		Nax2	48	0.00691
	Cfd60	67	0.00044		Wmc96	70	0.00029
	Barc299	68	0.0004o		Wmc18	70	0.00029
	Wmc96	67	0.00044		Xgwm335	70	0.00029
	Wmc18	67	0.00044		Cfd60	70	0.00029
	Xgwm335	67	0.00044		Barc299	69	0.00032
YP	Wmc504	85	0.00000	GMP	Wmc504	89	0.00000
	Wmc11	85	0.00000		Wmc11	89	0.00000
	Xgwm350	85	0.00000		Xgwm350	89	0.00000
	Xgwm205	85	0.00000		Xgwm205	89	0.00000
	Nax2	50	0.00597		Nac2	51	0.00540
	Wmc18	71	0.00024		Wmc96	69	0.00036
	Xgwm335	71	0.00024		Wmc18	69	0.00036
	Cfd60	71	0.00024		Xgwm335	69	0.00036
SSI	Wmc504	47	0.00871		Cfd60	69	0.00069
	Wmc11	47	0.00871		Barc299	73	0.00015
	Xgwm350	47	0.00871		Barc124-1	31	0.04427
	Xgwm205	47	0.00871	YSI	Wmc96	34	0.03338
STI	Wmc504	47	0.00871		Wmc18	34	0.03338
	Wmc11	47	0.00871		Xgwm335	34	0.03338
	Xgwm350	47	0.00871		Cfd60	34	0.03338
	Xgwm205	47	0.00871		Wmc661-2	30	0.04660
HM	Wmc504	40	0.01726		Wmc504	36	0.02562
	Wmc11	40	0.01726		Wmc503-1	35	0.02899
	Xgwm350	40	0.01726		Wmc11	35	0.02946
	Xgwm205	40	0.01726		Xgwm350	35	0.02946
MSTIk1	Wmc504	46	0.00889		Xgwm205	35	0.02946
	Wmc11	46	0.00889	SSPI	Wmc504	74	0.00011
	Xgwm350	46	0.00889		Wmc11	74	0.00011
	Xgwm205	46	0.02562		Xgwm350	74	0.00011
SMTIk2	Wmc504	80	0.00002		Xgwm205	74	0.00011
	Wmc11	80	0.00002		Nac2	48	0.00729
	Xgwm350	80	0.00002		Barc182-3	30	0.04637
	Xgwm340	37	0.02359		Wmc96	63	0.00089
	Xgwm205	80	0.00002		Wmc18	63	0.00089
	Nax2	37	0.02359		Xgwm335	63	0.00089

Table 6: The correlations among relative grain yields under salt stress (YS), non-stress (YP) and different tolerant salt indices.

	YS	YP	SSI	STI	TOL	HM	MSTIk1	MSTIk2	MP	GMP	SSPI	YSI
YS	1.00											
YP	0.988**	1.000										
SSI	0.707**	0.784**	1.000									
STI	0.968**	0.981**	0.765**	1.000								
TOL	0.847**	0.917**	0.900**	0.904**	1.000							
HM	0.997**	0.997**	0.748**	0.978**	0.885**	1.000						
MSTIk1	0.981**	0.994**	0.800**	0.991**	0.914**	0.990**	1.000					
MSTIk2	0.864**	0.848**	0.705**	0.887**	0.709**	0.858**	0.874**	1.000				
MP	0.996**	0.998**	0.754**	0.979**	0.890**	1.000**	0.991**	0.858**	1.000			
GMP	0.988**	0.993**	0.776**	0.989**	0.891**	0.993**	0.998**	0.884**	0.994**	1.000		
SSPI	0.832**	0.905**	0.885**	0.904**	0.997**	0.871**	0.904**	0.708**	0.876**	0.879**	1.000	
YSI	-0.513	-0.634*	-0.860**	-0.617	-0.887**	-0.576*	-0.628*	-0.397	-0.584	-0.585	-0.892**	1.000

*: significant at 95(%) level, **: significant at 99(%) level

The correlation between SSR marker data and nine indices:

The association analysis of the 24 SSR marker data YS, YP, and the nine indices (SSI, STI, HM, MSTIk1, MSTIk2, MP, GMP, YSI and SSPI) showed high determination coefficients (R2) between molecular markers and all indices (Table 5). Eighteen correlations of determination coefficients were found between fifteen different molecular loci and nine indices and ranged from 37 to 85(%) at a significance level 0.05. The most informative markers were wmc504, wmc11, xgwm350 and xgwm205. These four SSR markers were also cooperative and capable of explaining the variability of the YS, YP, SSI, STI, HM, MSTIk1, MSTIk2, MP, GMP, YSI and SSPI indices. The SSR markers, wmc504, wmc11, xgwm350 and xgwm205 were correlated with all indices,(SSI, STI, HM, MSTIk1, MSTIk2, MP, GMP, YSI and SSPI). Regarding the other markers, one locus of cslinkNax2SSR markers were correlated with YS, YP and four indices (SMTIk2, MP, GMP and SSPI). And, wmc18 and cfd60were correlated with YS, YP and three indices (MP, GMP and YSI) and, wmc18, was correlated with SSPI, also, and, barc299, was correlated with YS, and two indices (MP and GMP). These results indicated that the molecular markers were significantly associated with indices and that wmc504, wmc11, xgwm350 and xgwm205 were the best SSR markers for explaining the indices variability in wheat genotypes. Identical conclusion were reported for rice, where perfect, correlation was found between molecular markers and phenotypic traits concerning to salt tolerance (Kordrostami et al. 2016). Barakat et al. (2012) xgwm456, wmc25, wmc44, wmc94, wmc161, wmc273, wmc327 and xgwm566 were related to GFR under heat stress. Displayed that regression analysis for the relationship among the 12 SSR markers and the agronomic traits, were highly significant.

And R2 ranged from 8 to 64(%). The regression analysis for the relationship between the five SSR markers (barc32, barc52, barc76, barc80 and barc156 and, the four physiological traits under drought stress of wheat was highly significant. And R2 ranged from 15 to 39(%) reported by Barakact et al. (2015). In addition, Thiry et al. (2016) reported that, the determination coefficients (R2) were found between yield score stress index (YSSI) and yield under stress (YS) and ranged from 91 to 99(%). In addition, Moghaieb et al. (2011) reported that the Egyptian wheat cultivars, Beni-Suef, Sohag, and Gemmiza10 were distinguished by SSR primers. Barakat et al. (2016) found that ten SSR markers (barc242, barc228, barc286, barc149, barc232, barc109, barc57, barc77, wmc82 and wmc89) detected specific alleles only in the drought-tolerant genotypes and the determination coefficients (R2) were found 16, 56, 35, 16, 32, 44, 16, 23, 23 and 34 respectively, between spectral reflectance indices and ten markers. Results in the present investigation revealed that the cslinkNax2 marker gave the specific band at 280bp with the tolerant wheat genotypes (L2, L4 and L6), while it was absent in sensitive genotypes (Fig. 3 and Table 5). Appearing in the tolerant genotypes but not in sensitive genotypes, those fragments were found to be positive markers for salt tolerance. Two major genes for Na+ exclusion in wheat, gwm312 marker (Nax1) and cslinkNax2 marker (Nax2)., for the choice of Nax2 lines in segregating populations, development and evaluation of co-dominant marker, cslinkNax2 was used . The Nax2 has lowered the Na+ concentration in bread wheat amazingly because this gene is analogue to the TaHKT1; 5-D Na+ transporter actually found in bread wheat, putatively at the Kna1 locus (James et al., 2011). In a conclusion, if a cheerful marker band for salt tolerance in salt-sensitive genotypes, it can be discussed on the base that bands with same molecular sizes could signify amplified DNA fragments with different sequences. These fragments can be considered as a linked marker to salt tolerance.

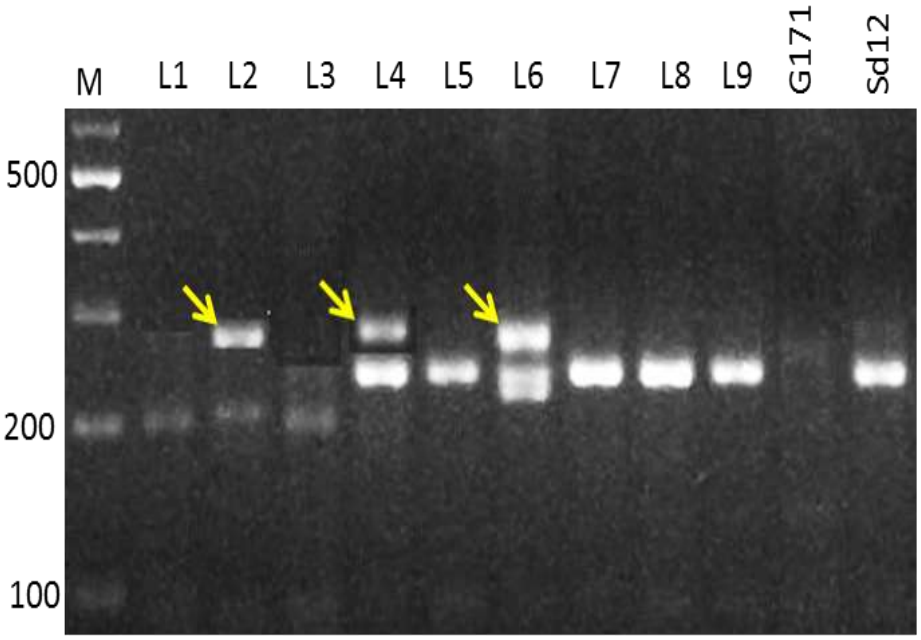

Figure 3: Agarose gel (3%) showing PCR products, cslinkNax2 primer from DNA of eleven wheat genotypes.

CONCLUSION

Conferring to results, salinity yield of some genotypes were tolerant to salt stress, demonstrating that enough genetic variability was current for salinity tolerance among the studied genotypes. Founded on correlation, we can say that HM, MP, GMP, MSTIk1 and STI indices were the greatest pointers of yield under stressed environment due to their significant correlations with Ys. Using HM, MP, and GMP, MSTIk1 and STI indices, genotypes can be chosen suitably under stressed environments. Datum on the markers providing tools for breeders to make use of these genes is obtainable to all concerned. These genotypes are being evaluated on saline sites.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support. Great appreciations are also due to Prof. Shaalan, M.I. And Prof. Motawei, M. for their suggestions and proofreading during the preparation of this manuscript.

AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

REFERENCES

Ahmad M, Shahzad A, Iqbal M, Asif M, Hirani AH. (2013). Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. Australian Journal of Crop Science.7:66.

Ahmad M. (2011). Evaluation of wheat genotypes for salt tolerance based on conventional and

molecular approaches (Doctoral dissertation, Ph.D Thesis, Department of Plant Breeding and Genetics, PirMehr Ali Shah Arid Agriculture. University, Rawalpindi, Pakistan).

Al-doss AA, Elshafei AA, Moustafa KA, Barakat MN (2011). Comparative analysis of diversity based on morpho- agronomic traits and molecular markers in durum wheat under heat stress. J. Biotechnol., 10, 3671-3681.

Al-Murish TM, Elshafei AA, Al-Doss AA, Barakat MN. (2013). Genetic diversity of coffee (*Coffee arabica* L.) in Yemen via SRAP, TRAP and SSR markers. J. Food Agric. Environ., 11, 411-416.

Ammar MH, Alghamdi SS, Migdadi HM, Khan MA, El-Harty EH, Al-Faifi SA (2015). Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers. Saudi J. Biol. Sci., 22(3), 340-350.

Anwar, J, Subhani, G. M., Hussain, M., Ahmad, J., Hussain, M., & Munir, M. (2011). Drought tolerance indices and their correlation with yield in exotic wheat genotypes. Pak. J. Bot, 43, 1527-1530.

Ashraf MY, Akhter K, Hussain F, Iqbal J. (2006). Screening of different accessions of three potential grass species from cholistan desert for salt tolerance. Pakistan J. Bot. 38(5 SPEC. ISS.): 1589–1597.

Ashraf M, Foolad, MR. (2013). Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection (R Tuberosa, Ed.). Plant Breeding 132(1): 10–20.

Barakat MN, Al-doss AA, ElshafeAA, Moustafa K.A. (2012). Bulked segregate analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers. 11(61): 12436–12442.

Barakat MN, Moustafa KA, Elshafei AA, Salem AK. (2015). Identification of QTLs for four physiological traits in an advanced backcross population of wheat under drought stress. 8(2): 122–129.

Barakat M, El-Hendawy S, Al-Suhaibani N, Elshafei A, Al-Doss A, Al-Ashkar I, Ahmed E, Al-Gaadi K. (2016). The genetic basis of spectral reflectance indices in drought-stressed wheat. Acta Physiol. Plant.38(9).227-237.

Bouslama, M, Schapaugh WT. (1984). Stress Tolerance in *SoY Peans*. I. Evaluation of Three Screening Techniques for Heat and Drought Tolerance1. Crop Science 24(5): 933.

Byrt CS, Platten J D, Spielmeyer W, James Lagudah RA, Dennis ES, Tester M, Munns R (2007). HKT1; 5-like cation transporters linked to Na+ exclusion loci in wheat, Nax2 and Kna1.Plant Physiology, 143(4), 1918-1928.

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W. (2002). Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat.Theor. Appl. Genet., 104, 399-407.

Farshadfar E, Sutka J (2002). Multivariate analysis of drought tolerance in wheat substitution lines. Cereal Res. Commun. 31: 33-39.

Federer WT, King F. (2006). Variations on Split Plot and Split Block Experiment Designs.

Fernandez GCJ (1992). Effective selection criteria for assessing stress tolerance. Tainan. Taiwan.

Fischer R, Maurer R (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res., 29, 897-912.

Gomez KA and Gomez AA. (1984). Statistical procedures for agricultural research John Wiley & Sons.

Hammer D, Harper A, Ryan P. (2001). PAST: Palaeontological Statistics software package for education and data analysis. Palaeontol.Electron., 4-9.

Hassanein AMA, Al-Soqeer AA. (2018). Morphological and genetic diversity of Moringaoleifera and Moringaperegrina genotypes. Horticulture, Environment, and Biotechnology: 1-11.

Hosseini, S.J., Z.T. Sarvestani, and H. Pirdashti. (2012). Analysis of Tolerance Indices in Some Rice (Oryza sativa L .) Genotypes at Salt Stress Condition. 3(1): 1–10.

Huang S, Spielmeyer W, Lagudah ES, Munns R, (2008). Comparative mapping of HKT genes in wheat, barley, and rice, key determinants of Na+ transport, and salt tolerance. Journal of experimental botany, 59(4), 927-937.

James, RA, Blake C, Byrt CS, Munns R (2011). Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1; 4 and HKT1; 5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. Journal of Experimental Botany, 62(8), 2939-2947.

Katerji, N, Van JW, Hamdy A, Mastrorilli M (2000). Salt tolerance classification of crops

according to soil salinity and to water stress day index. Agric. Water Manag. 43(1): 99–109.

Khierallah H, Bader , Baum M, Hamwieh A (2011). Assessment of genetic diversity for some Iraqi date palms (Phoenix dactylifera L.) using amplified fragment length polymorphisms (AFLP) markers. Afr. J. Biotechnol., 10, 9570-9576.

Klute A, Richard RW, Weaver W, Mickelson SH, Sparks D.L, Bartels JM, Dane JH, Topp GC, Ulery AL, Drees LR (1994). Methods of soil analysis. Soil Science Society of America.

Kordrostami M, Rabiei B, Kumleh HH (2016). Association analysis, genetic diversity and haplotyping of rice plants under salt stress using SSR markers linked to SalTol and morpho-physiological characteristics. Plant systematics and evolution 302: 871-890.

Kumar S, Kumar V, Kumari P, Singh AK, Singh R (2016). DNA fingerprinting and genetic diversity studies in wheat genotypes using SSR markers. J. Environ. Biol., 37-319.

Lindsay, MP, Lagudah ES, Hare, R A, Munns R. (2004). A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. Functional Plant Biology, 31(11), 1105-1114.

Lindsay MP, Spielmeyer W, Lagudah E, James R, Munns R, Huang S. (2008). Markers for salinity tolerance in wheat plants and the use thereof in breeding programs. U.S. Patent Application No AU2005251389

Manly K.F, Cudmore Jr, Meer R.H (2001). Map Manager QTX, cross-platform software for genetic mapping. Mammalian Genome 12: 930-932.

Mardi M, Naghav MR, Pirseyedi SM, Alamooti MK, Monfared SR, Ahkami AH, Omidbakhsh MA, Alavi NS, Shanjani PS, Katsiotis A (2011). Comparative assessment of SSAP, AFLP and SSR markers for evaluation of genetic diversity of durum wheat (*Triticum turgidum L*. var. durum). J. Agr. Sci. Tech., 13, 905-920.

Mitra J. (2001). Genetics and genetic improvement of drought resistance in crop plants. Current Science 80(6): 758–763.

Moghaieb REA, Abdel-Hadi AHA, Talaat NB. (2011). Molecular markers associated with salt tolerance in Egyptian wheats. Afr. J. Biotechnol., 10, 18092-18103.

Moosavi SS, Yazdi SB, Naghavi MR, Zali AA, Dashti H, Pourshahbazi A (2008). Introduction of new indices to identify relative drought tolerance and resistance in wheat genotypes. Desert, 12, 165-178.

Negrão S, SM, Schmöckel, M, Tester (2017). Evaluating physiological responses of plants to salinity stress. Annals of Botany 119(1): 1–11.

Nei M, Li WH. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76(10): 5269–5273.

Nouri A, Etminan A, Teixeira da, Silva JA, Mohammadi R (2011). Assessment of yield, yield-related traits and drought tolerance of durum wheat genotypes (Triticumturgidum var. durum Desf.). Aust. J. Crop Sci., 5 (1), 8-16.

Papathanasiou F, Dordas C, Gekas F, Pankou C, Mylonas I, Tsantarmas K, Sistanis I. (2015). The use of stress tolerance indices for the selection of tolerant inbred lines and their correspondent hYPrids under normal and water-stress conditions. Procedia Environ. Sci. 29(Agri): 274–275.

Plaschke J, Ganal MW, RoKder MS (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers.Theor. Appl. Genet., 91, 1001-1007.

Prasad M, Varshney RK, Balyan HS, Gupta PK, Roy JK. (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Genet.,100, 584-592.

Rosielle AA, Hamblin J. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci., 21(6), 943-946.

Sanjari PA, Yazdansepas A. (2008). Genotypic variation of the trait stem reserves in bread wheat (*Triticum aestivum L*.) genotypes under post anthesis drought stress condition. Iran. J. Field Crop Sci., 39, 181-191.

Schneider KA, Rosales R, Ibarra F, Cazares B, Acosta JA., Ramirez P, Wassimi N, Kelly JD. (1997). Improving common bean performance under drought stress. Crop Science 37(1): 43–50.

Shahzad A, Ahmad M, Iqbal M, Ahmed I, Ali GM. (2012). Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers.Genet. Mol. Res. 11, 679-692.

Shahzad A. (2007). Biochemical markers for screening wheat for salt tolerance.Ph. D. dissertation, Institute of Soil and

Environmental Sciences University of Agriculture Faisalabad.

Singh Ak. (2004). The Physiology of Salt Tolerance in Four Genotypes of Chickpea during Germination. 6: 87–93.

Singh S, Sengar RS, Kulshreshtha N, Datta D, Tomar RS, Rao VP, Garg D, Ojha A. (2015). Assessment of Multiple Tolerance Indices for Salinity Stress in Bread Wheat (Triticumaestivum L.). Journal of Agricultural Science 7(3): 49-59.

Smith JSC, ECL, Chin H, Shu OS, Smith SJ, Wall ML Senior SE, Mitchell S, Kresovich, Ziegle J. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): Comparisons with data from RFLPS and pedigree. Theoretical and Applied Genetics 95(1–2): 163–173.

Stavridou E, Hastings A, Webster RJ, Robson PRH. (2017). The impact of soil salinity on the yield, composition and physiology of the bioenergy grass Miscanthus ×giganteus. GCB Bioenergy 9(1): 92–104.

Steel RGD, Torrie JH. (1980). Principles and Procedures of Statistics: A Biometrical Approach. .2nd Ed. McGraw-Hill Publishing Co., New York. : 631p.).

Swofford DL. (2001). PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b5.

Talebi R, Fayaz F, Naji AM. (2009). Effective selection criteria for assessing drought stress tolerance in durum wheat (Triticum durum Desf.). Gen. Appl. Plant Physiol., 35, 64-74.

Thiry AA, Chavez PN, Reynold MP, Davies WJ. (2016). How can we improve crop genotypes to increase stress resilience and productivity in a future climate? A new crop screening method based on productivity and resistance to abiotic stress. Journal of Experimental Botany 67(19): 5593–5603.

Zarei L, Farshadfar E, Haghparast R, Rajabi R, Mohammadi M, Sarab B. (2007). Evaluation of some indirect traits and indices to identify drought tolerance in bread wheat (*Triticum aestivum L*.). Asian Journal of Plant Sciences 6(8): 1204–1210.

Zeynali ME, Rabii A, Baharvand H (2004). Synthesis of partially hYProlyzed polyacrylamide and investigation of solution properties (viscosity behaviour). Iran. Polym. J., 13(6), 479-484.

Zhu M, Shabala S, Shabala L, Fan Y, Zhou M.X (2016). Evaluating Predictive Values of Various Physiological Indices for Salinity Stress Tolerance in Wheat. Journal of Agronomy and Crop Science 202(2): 115–124.