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Morphological variability and genetic diversity of wheat genotypes grown on saline soil and identification of new promising molecular markers associated with salinity tolerance

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ABSTRACT

Eleven bread wheat genotypes grown at Siwa Oasis and Ashmon, Menofeya Governorate, during the winter season of 2016/2017 were examined for their agronomic traits under salinity stress. Owing to the differences in the salinity levels at the two locations, significant differences were noticed among the tested genotypes for all traits. Lines L2 and L4 showed the highest grain yields, whereas L3 showed the lowest. The most suitable parameters for screening stress-tolerance were tolerance indices and high-yielding potentiality. In this investigation, 33 SSR (Simple Sequence Repeat) primers led to the determination of one to three alleles per primer, with an average of 1.36. The use of 31 EST (Expressed Sequence Tag)-SSR markers led to the determination of 38 polymorphic alleles, ranging from one to five, with a mean of 1.23 per locus. A cluster analysis using the SSR and EST-SSR information divided the 11 wheat genotypes into three groups.

Abbreviations: AFLP: Amplified Fragment Length Polymorphism; **DP:** Discrimination Power; **EST:** Expressed Sequence Tag; **PCA:** Principal Coordinate Analysis; **PCR:** Polymerase Chain Reaction; **PIC:** Polymorphic Information Content; **SRAP:** Sequence Related Amplified Polymorphic; **SSR:** Simple Sequence Repeat; **UPGMA:** Unweighted Pair Group Method With Arithmetic Average.

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Salt tolerance; agronomic traits; SSR markers; EST-SSR markers; wheat breeding

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally. Furthermore, wheat is the largest food source in Egypt despite a 49% gap between wheat consumption and production (Ouda and Zohry 2017). One of the challenges in filling the gap between wheat production and consumption in Egypt is the problem of salinization in the Nile Delta. For example, 33% of the agriculture area in Egypt is salinized owing to low precipitation (<25 mm annually), drain water re-use, and the constraints that policymakers put on the cultivation of rice (Bortolini et al. 2018).

Wheat response to salinity stress is a multiplex phenomenon which involves changes in several morphological and physiological traits, in addition to affecting several biochemical pathways. Salinity stress inhibits plant growth by increasing water deficits, ion toxicity, ion imbalance, and nutritional and hormonal imbalance (Ashraf and Foolad 2013; Acosta-Motos et al. 2017; Ismail and Horie 2017). Plant responses to counter the salt-induced adverse effect include ion exclusion, accumulation of organic osmolytes, antioxidants production, and changes in mineral and nutrients uptake (Ismail and Horie 2017; Rahneshan et al. 2018). Therefore, developing salinity stress tolerant genotypes requires a profound understanding of several physiological and morphological mechanisms of plant response to salinity stress to successfully select salinity stress-tolerant genotypes. Phenotypic traits such as growth, water relation, ion homeostasis, photosynthesis, yield components, and senescence have been used to directly select for salinity stress tolerant genotypes (Negrão et al. 2017).

Molecular markers offer a contemporary solution to improve the selection efficiency of complicated traits such as salinity stress. The main applications of molecular markers can be classified into two categories: (a) assessment of genetic diversity and (b) identification and characterization of genomic regions controlling the trait of interest (Guichoux et al. 2011; Li et al. 2012; Postolache et al. 2014; Meyer et al. 2017; Ishikawa et al. 2018). Among the several molecular markers platforms available, such as Single Nucleotide Polymorphism and genotype by sequencing (Cavanagh et al. 2013; Ishikawa et al. 2018) Microsatellites (simple sequence repeat [SSR]) were used in the this study. The SSR marker platform, which can be developed using genomes (gSSRs) or transcriptomes (EST-SSRs), is a powerful tool for examining population genetic diversity (Meyer et al. 2017) and marker-assisted selection (Gadaleta et al. 2011). Furthermore, EST-SSR primers have generally been effective as strong molecular genetic tools in a huge number of cereal crop species because of their high level of transferability across species, close association with functional genes, and recognition of variation in coding sequences. Moreover, EST-SSRs were found to be more conservative than SSRs (Li et al. 2012;

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Postolache et al. 2014). Even though the EST-SSR markers produced high-quality information, they are less polymorphic than the gSSR markers (Ren et al. 2017). Generally, SSR markers (EST-SSR and gSSR) are suitable for parental identification, pedigree analysis, and development of newly improved genotypes as they are independent of environmental conditions and plant development stage. Thus, SSR markers are a useful tool to indirectly select for desirable alleles or traits if a tight linkage relationship is established between the SSR marker and the gene controlling the trait (El Siddig et al. 2013).

In this study, two locations, Siwa Oasis (located in the north-west of the western desert of Egypt) and Ashmon (located in northern Egypt) were used to evaluate the impact of soil salinity stress on several aspects of wheat plants under field conditions. Siwa Oasis was selected in this study because of its promising capabilities for agricultural development. However, soils in this Oasis suffer from the problem of soil salinity which resulted in a deleterious effect on wheat productivity. Moreover, the Ashmon location was considered a control location with no soil or water problems. The main objective in using these two locations was to compare the performance of the studied materials grown under normal growth conditions with that grown under saline soil conditions. Two check cultivars and nine recently developed and potentially salinity stress-tolerant lines were evaluated at the Siwa and Ashmon locations. The main aims of this study were to:

(1) characterize the performance of the studied materials under saline and normal growth conditions,
(2) use phenotypic performance, gSSR, and EST-SSR to investigate genetic variability among the studied materials, and
(3) assess the effectiveness of gSSR and EST-SSR in identifying salinity stress-tolerant genotypes.

Materials and methods

Plant materials and field conditions

Eleven wheat genotypes were used in the current study, i.e. two recently released cultivars ('Giza171' and 'Sids12') and nine elite lines, 'L1' to 'L9', potentially tolerant to salinity stress. Information about seed sources, selection history, and pedigrees are presented in the supporting information (Table 1).

Table 2. Soil physical analysis of the two experimental sites at Ashmon – Menofya, and Siwa Oasis during 2016/2017 growing season.

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

The studied materials were planted at two locations; the Experimental Farm of the Desert Research Center located in Siwa Oasis (29°00′12″N 26°00′3″ E) and a Grower farm located in Ashmon (30°18′16″ N 31°2′5″ E) in the last week of November during the 2016/2017 growing season. Siwa is an urban oasis in Egypt, bordering Libya, and 560 km from Cairo; while, Ashmon is located in the middle delta, 42 km from Cairo (northern Egypt). Soil samples were collected (0–30 cm depth) during November directly before planting and analyzed according to Klute et al. (1994). The main soil physical and chemical characteristics are shown in Tables 2 and 3 (supporting information). For each location, a randomized complete block design was used with three replicates. The 11 genotypes were assigned randomly to each plot within each replicate and location. The plot sizes were 3.5 m long and 15 rows wide with 20 cm between rows. Standard agronomic practices of fertilization and irrigation schedules, for each location, were followed.

Phenotypic measurements

The following variables were measured at both locations, i.e. in stressed and non-stressed environmental conditions. Plant height (cm) of five randomly selected plants was measured after physiological maturity in each plot as distance from the soil surface to the tip of the spike, excluding awns. Random samples of 10 plants were collected from each plot directly before harvest. The collected samples were dried at 70°C for 72 h. Then, the number of spikes per plant, seed number per spike, spike length, spikelets number per spike, and grain number per plant were determined on the dry samples. All plants in each plot were cut at 5 cm above the soil surface and left to dry in the middle of the plots. After three days, plants from each plot were threshed separately using a locally made single plot thresher, in which seeds and straw were collected, paged, numbered, and then dried and weighed.

Molecular analysis

Molecular analysis was conducted at the Biotechnology Lab, Department of Plant Production, Food and Agriculture

Table 1. 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.

Table 3. 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 (0–30 cm)											
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

Sciences, King Saud University, Riyadh, Saudi Arabia. DNA was extracted from wheat genotypes using the Wizard Genomic DNA purification Kit (Promega Corporation Biotechnology, Madison, WI, USA). Then, the extracted DNA was treated with RNase and stored in a refrigerator at −20°C. Before conducting the EST-SSR and SSR analysis, DNA was diluted to 25 ng/µL.

Forty-six EST-SSR primers (Peng and Lapitan 2005) and 50 SSR primers (Somers et al. 2004) were used (supplementary data Table 6a and 6b). The PCR mixture comprised 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 EST-SSR and SSR analyses involved a primary denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50, 52, 55, and 60°C (dependent on EST-SSR and 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 0.1 µg cm^{-3} ethidium in TBE buffer. After electrophoresis, a photograph of the gel was captured using a UV trans-illuminator. The EST-SSR and SSR data were scored on the basis of presence (1) or absence (0) of a given marker, after excluding unreproducible bands.

Statistical analysis

Phenotypic data

Analysis of variance was performed using SAS® v9.2 (2008, SAS Institute Inc., Cary, NC, USA), following the linear model (Federer and King 2007):

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

where, Y_{ijm} is the response measured on the $_{ijm}$ plot, μ is the overall mean, E_i is the effect of the ith Environment (two locations), $EB_{(i)j}$ is the jth block nested within the ith environment, G_m is the effect of the mth genotype, EG_{im} is the interaction effect among the ith environment, and Gth genotype, and εijm 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 the Bartlett's Test (Steel and Torrie 1980).

Molecular marker data and genetic variability

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

$$\mathbf{SM} = 2N_{ij}/(N_i + N_j)$$

where, N_{ij} is the number of alleles present in both the ith and jth genotypes, N_i is the number of bands present in the ith genotype, and N_j is the number of alleles present in the jth genotype.

The similarity matrix was then subjected to the rate unweighted pair group method with arithmetic average (UPGMA) grouping 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 version 1.62 (Hammer et al. 2001). Furthermore, to ensure the reliability of the generated dendrogram, 1000 simulations were performed using PAUP* version 4.0.b5 (Swofford 2001). Polymorphic information content (PIC) was calculated as follows (Smith et al. 1997):

$$\mathbf{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

where, p_i is the frequency of the ith allele across genotypes.

To identify the informative markers, and study the correlation between genetic diversity and average grain yield for each genotype in the stressed environment, the association analysis between molecular data of the nine SSR and EST-SSR markers was performed using Map Manager QTX, vQTXb20 (Manly et al. 2001).

Results

Mean performance of yield and its components

Highly significant differences were observed among genotypes tested for all studied traits (Table 4). The results showed that the highest number of spikes per plant was obtained in genotype L6 (3.81) followed by L2 (3.40), while the lowest number of spikes per plant was obtained in genotype L9 (1.82). The highest number of grains per plant was obtained for genotype L2 (177.3), while the highest values in grain yield, straw yield, number of grains per plant, 1000-grain weight, and number of spikes per plant were obtained in genotype L2.

The mean performance combined over the two environments (stress and adequate conditions), as well as under each environment for yield and associated components of the 11 bread wheat genotypes examined are shown in Table 4. It is noteworthy that the mean performances of all genotypes under adequate conditions were higher than those under salt stress environment for all recorded traits. However, the differences were significant in only straw yield. For plant height, the genotypes L5 and G171 were the tallest genotypes under stress, while L7 was the shortest under both adequate and stress conditions. The number of spikes per plant was the highest in genotype L6 under stress and adequate conditions, as well as in the combined data (3.57, 4.06, and 3.81, respectively). The lowest values for these traits were observed in genotypes L9 and L3 under

Table 4. Mean performance combined over the two environments (under stress and adequate) as well as under each environment for yield and its components of eleven bread wheat genotypes tested.

Genotypes	Plant height (cm)	Spikes no./Plant	Spike length (cm)	Spikelets no/spike	1000-grain weight(g)	Grains no./ plant	Grain yield (g)	Straw yield (g)
Adequate environment	106.59	2.81	17.12	20.67	32.43	123.89	3.34	8.28
Salt stressed environment	103.10	2.54	16.78	19.23	29.64	107.40	2.87	6.39
LSD (5 %)	NS†	NS†	NS†	NS†	NS†	NS†	NS†	0.37
Combined data over environments								
(L1)	91.78	2.43	14.64	19.11	29.37	100.87	2.90	7.11
(L2)	95.57	3.40	20.08	21.59	40.74	177.53	4.78	10.65
(L3)	113.12	2.02	18.37	22.18	22.59	101.22	2.07	4.92
(L4)	104.88	3.07	15.18	22.17	35.61	164.08	4.31	9.16
(L5)	121.88	2.54	20.99	16.61	35.30	95.26	2.91	7.06
(L6)	105.82	3.81	15.05	22.15	34.54	137.00	3.91	10.53
(L7)	96.12	2.58	14.43	18.84	21.02	106.96	2.67	6.16
(L8)	95.33	2.59	15.63	18.73	23.91	131.06	3.21	6.70
(L9)	97.29	1.82	16.08	21.81	35.21	81.31	2.09	4.94
Sids12	108.21	2.52	19.20	18.42	26.23	108.48	2.70	7.05
GIZA 171	123.29	2.60	16.81	17.85	36.88	88.31	2.63	6.42
LSD (5 %)	7.63	0.28	2.15	1.52	8.00	15.80	0.57	1.06
Adequate environment								
(L1)	92.07	2.48	14.61	19.61	29.71	107.23	3.03	7.54
(L2)	96.73	3.73	20.46	22.27	47.30	167.73	5.23	11.90
(L3)	117.00	2.13	19.09	23.28	23.11	109.77	2.20	4.91
(L4)	105.17	3.26	15.26	23.46	39.37	184.87	4.74	10.08
(L5)	125.13	2.68	21.42	17.51	34.62	112.47	3.22	8.25
(L6)	109.40	4.06	15.63	23.21	36.07	147.67	4.30	13.52
(L7)	96.37	2.73	14.33	19.24	20.38	106.73	2.86	6.55
(L8)	98.23	2.69	15.86	19.56	25.08	140.83	3.47	7.22
(L9)	98.23	1.89	15.89	22.66	37.31	86.07	2.23	5.45
Sids12	110.60	2.65	19.40	18.66	26.55	111.17	2.79	8.34
GIZA 171	123.53	2.59	16.39	17.93	37.17	88.27	2.70	7.28
LSD 5%	12.38	0.35	3.38	2.47	12.49	28.22	1.05	1.69
Salt stressed environment								
(L1)	91.49	2.38	14.67	18.61	29.02	94.50	2.77	6.68
(L2)	94.41	3.06	19.70	20.90	34.17	147.33	4.32	9.40
(L3)	109.23	1.92	17.65	21.08	22.06	92.67	1.94	4.92
(L4)	104.59	2.89	15.10	20.88	31.85	143.28	3.88	8.23
(L5)	118.63	2.41	20.56	15.72	35.97	78.06	2.60	5.87
(L6)	102.24	3.57	14.47	21.09	33.01	126.34	3.52	7.54
(L7)	95.88	2.42	14.53	18.44	21.66	107.19	2.48	5.77
(L8)	92.43	2.49	15.41	17.89	22.74	121.29	2.96	6.18
(L9)	96.35	1.75	16.26	20.96	33.11	76.56	1.94	4.42
Sids12	105.81	2.40	18.99	18.18	25.90	105.78	2.61	5.75
GIZA 171	123.05	2.61	17.22	17.78	36.58	88.36	2.56	5.55
LSD 5%	9.73	0.45	2.89	1.93	10.80	16.33	0.50	1.40

†NS, nonsignificant at the 0.05 probability level.

stress. For 1000-grain weight (g), L2 showed the highest values under adequate conditions, as well as in the combined data, while the check variety G171 and L5 showed the highest 1000-grain weight under stress conditions (36.58 and 35.97, respectively). In contrast, L7 and L3 showed the lowest 1000-grain weight under stress and adequate conditions, as well as in the combined data. Genotype L2 produced the maximum number of grains per plant under stress as well as in the combined data, while L4 produced the maximum number of grains per plant under adequate conditions. The lowest values for this trait were observed in the check variety G171. Regarding grain yield per plant, both L2 and L4 produced the heaviest grains, while L3 and L9 produced the lightest ones. Six of the nine selected lines surpassed both the check varieties (Sids12 and Giza171) in grain yield per plant in the combined data (L1, L2, L4, L5, L6, and L8). These genotypes were selected for continuation in the wheat breeding program. For straw yield per plant (g), both L2 and L6 recorded the highest values under adequate conditions, as well as in the combined data, while L2 and L4 recorded the highest values under stress conditions. Generally, grain yield superiority for each of these genotypes was attributed to the high potentiality of two or more of the yield attributes (Table 4). Therefore, these genotypes should be further tested under different environments (years, locations, and cultural practices) in subsequent breeding program investigations to ensure their stability and better grain quality under such stress conditions.

SSR and EST-SSR combined analysis

Genetic diversity of molecular markers

Of the 96 different SSR and EST-SSR primer pairs used in this study, only 64 generated polymorphisms among the 11 wheat genotypes. A cluster analysis was performed based on similarity coefficients generated from the SSR and EST-SSR combinations data of the 83 scored bands. The cluster analysis grouped the 11 wheat genotypes into three main groups with similarity coefficients ranging from 0.32 to 0.70 with an average of 0.61. The maximum genetic similarity (0.70) was observed between genotypes L4 and L6 and the minimum (0.32) was between Sids12 and L3 (Table 5 and Figure 1). The first group supported by a bootstrap value of 29% contained four genotypes, (L7, L9, L8, and G171), that were salt-sensitive genotypes. The second group supported by a bootstrap value of 9% contained five wheat genotypes comprising two subgroups. The first subgroup supported by a bootstrap value of 67% included genotypes L4, L6, L1,

Table 5. The levels of genetic diversity in eleven wheat genotypes by SSR and EST- SSR markers.

				Allele/ Loci		Similarity coefficient		PIC value	
Source of primers	No. of primers	No. of loci	No. of alleles	Range	Average	Range	Average	Range	Average
SSR	50	33	45	1–3	1.36	0.19–0.7	0.46	0–0.987	0.203
EST-SSR	46	31	38	1–5	1.23	0.22–0.882	0.575	0 to.740	0.08
Combinations	96	64	83	1–5	1.29	0.32–0.7	0.6113	–	–

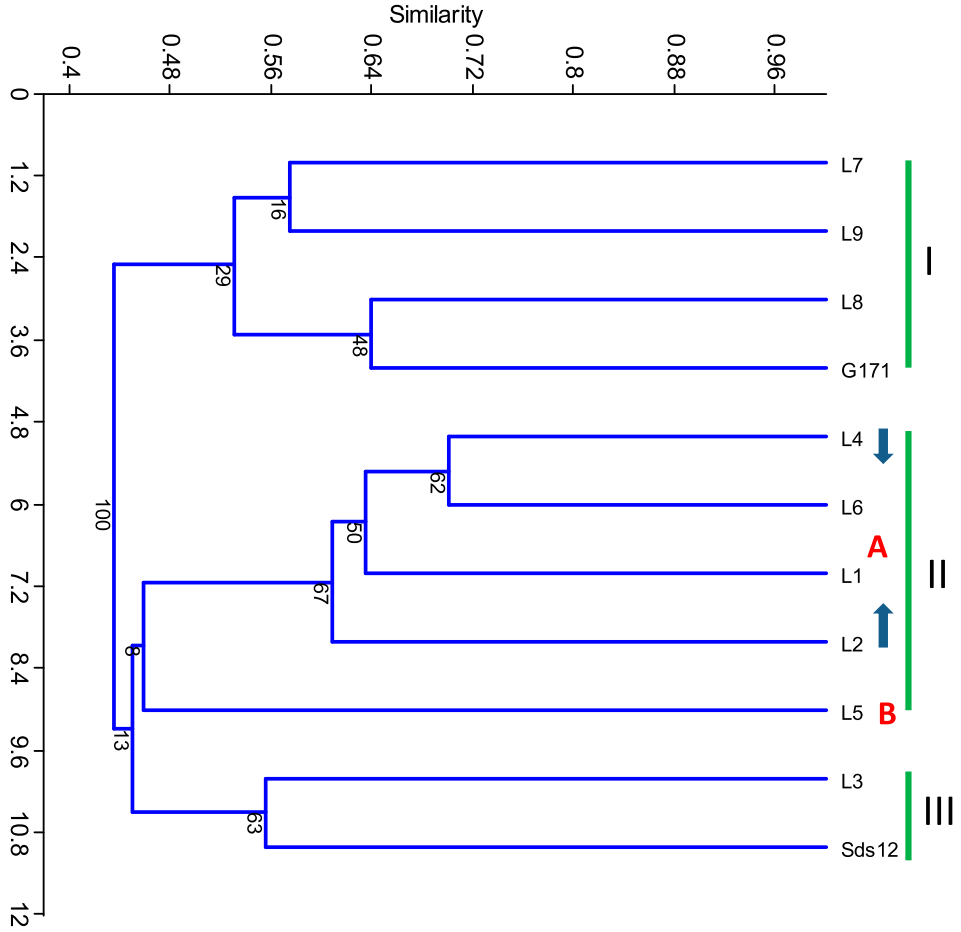

Figure 1. Unweighted pair group method arithmetic average (UPGMA) dendrogram for eleven wheat genotypes based on the allelic data of 33 SSR and 31 EST-SSR combinations.

and L2 that were salt-tolerant. The second subgroup supported by a bootstrap value of 8% included the L5 genotype that was salt-sensitive. The third cluster contained two wheat genotypes, (L3 and Sids12), that were salt-sensitive and supported by a bootstrap value of 63%.

Cluster analysis efficiently grouped the salt tolerant and sensitive genotypes separately into three clusters (Figure 1). The first and third groups contained all the salt-sensitive genotypes, while the second group contained all the salt-tolerant genotypes except genotype (L5), which was separated alone as a subgroup. These results recommended that cluster analysis could be used to distinguish between the salt-tolerant and sensitive genotypes.

Genetic information produced by SSR and EST-SSR markers

Thirty-three SSR markers were used to examine their discrimination power (DP) by calculating the PIC of their loci. A total of 45 bands (alleles) were amplified among the 11 wheat genotypes using 33 SSR markers. The number of amplified bands (alleles) per primer ranged from one allele, for the barc13 primer, to three alleles, for the barc5 primer, with a mean value of 1.36 alleles (Table 5). The sizes of the amplified alleles varied between 100 and 600 bp. The level of polymorphism among the 11 genotypes was estimated by calculating the PIC values for each of the 33 SSR loci. The PIC values varied greatly for all SSR loci tested. Twenty-two SSR primers detected a single allele, and their PIC values were zero. The PIC values of the remaining 11 primers ranged from 0.16 (barc63) to 0.92 (barc11) (Table 5). The PIC values were positively correlated ($r = 0.785$) with the number of amplified alleles per marker.

Thirty-one EST-SSR primers were used to investigate their DP by calculating their PIC. A total of 38 alleles were amplified among the 11 wheat genotypes, using 31 EST-SSR primers. The number of amplified bands (alleles) per primer ranged between one allele, for primer 'Xcwem3', to five alleles, for primer 'Xcwem54', with a mean value of 1.2 alleles (Table 5). The sizes of amplified alleles varied between 100 and 650 bp. The levels of polymorphism among the 11 genotypes were evaluated by calculating the PIC values for each of the 31 EST-SSR loci. The PIC values varied greatly for all EST-SSR loci tested. Twenty-eight primers detected a single allele and their PIC values were zero. The PIC values of the

Table 6. Specific SSR and EST-SSR markers in eleven wheat genotypes under salt stress.

Source of primers	Salt category	Markers	Chromosome number	Size (bp)	Genotypes	Coefficient of determination (R^2)	Significance level
SSR	Salt tolerant	Barc63	6A	210	L6	65	0.00114
		Barc124	5B	100	L1	41	0.01656
		Barc125	7D,3D,4B,5A	130	L1, L2, L4, L6, L8	63	0.00097
		Barc144	5D,5A	250	L2	41	0.01656
		Barc210	1D,2B	200	L2	41	0.01656
EST-SSR	Salt tolerant	Xcwem9	1D,1A,3A	390	L2, L4, L6	78	0.00004
		Xcwem40	5B,5A	120	L2, L4	65	0.00067
		Xcwem45	5B	225	L2, L6	47	0.00780
		Xcwem54	7A,1B,2B	220	L1, L2, L4, L6	63	0.00097

remaining three primers ranged from 0.165 (Xcwem11) to 0.742 (Xcwem54) (Table 5). The PIC values were positively correlated ($r = 0.963$) with the number of amplified alleles per primer. The present analysis indicated that the polymorphism revealed by EST-SSR primers was lower than that by gSSR markers. It could effectively differentiate diverse genotypes (Table 5).

Identification of new promising molecular markers linked with salinity stress tolerance

The SSR and EST-SSR primers generated in this study to identify salt tolerance-associated DNA markers are presented in Table 6. The SSR and EST-SSR analyses showed that some wheat genotypes were salt-tolerant (based on the origin of their field performance). The SSR markers barc63, barc124, barc144, and barc210 produced DNA bands of approximately 210, 100, 250, and 200 bp, respectively, that were present in the salt-tolerant genotypes (L6, L1, L2, and L2), but were absent in the salt-sensitive genotypes (L3, L5, L9, L7, and G171) (Table 6). The SSR marker barc125 (130 bp) was present in four salt-tolerant genotypes, (L1, L2, L4, and L6), but was absent in the salt-sensitive genotypes, (L3, L5, L9, L7, and G171) (Table 6). In this study, five markers (barc63, barc124, barc125, barc144, and barc210) were amplified in the salt-tolerant genotypes. These markers could explain 65, 41, 63, 41, and 41%, respectively, of phenotypic variation.

For the EST-SSR analysis, the polymorphic DNA fragment (390 bp) of primer Xcwem9 was identified in the salt-tolerant genotypes (L2, L4, and L6) but was absent in the salt-sensitive genotypes (L3, L5, L9, L7, and G171) (Table 6). The Xcwem54 (220 bp) marker was identified in the salt-tolerant genotypes (L1, L2, L4, and L6), but was absent in the salt-sensitive genotypes (L3, L5, L9, L7, and G171). The EST-SSR markers, Xcwem9 and Xcwem54, explained 78 and 63%, respectively, of the phenotypic variation. These markers appeared to be linked to the salt tolerance genotypes. In addition, the specific DNA bands generated from EST-SSR markers could be used to differentiate between the salt-tolerant (L1, L2, L4, and L6) and salt-sensitive (L3, L5, L9, L7, and G171) genotypes.

Discussion

The highest values of grain yield, straw yield, number of grains per plant, 1000-grain weight, and number of spikes per plant were obtained in genotype L2. These results are consistent with those reported by Afiah (2002) and Afiah et al. (2018). These results are also consistent with the reports of Shpiler and Blum (1990), who found that the number of kernels/spike was the most effective component of grain yield. These variations among genotypes might reflect, partially, their different genetic backgrounds. El-Hendawy et al. (2005) reported that spikelet number on the main stem decreased to a greater extent with salinity than spike length, grain number, and 1000-grain weight at maturity. Moreover, Ouda et al. (2006) reported that the level of wheat yield reduction, as a result of stress, was affected by genotypes.

The findings of Prasad et al. (2000) supported that the cluster obtained based on the similarity matrix, using the UPGMA algorithm, grouped the above cultivars into two main groups (I and II), each with two sub-groups (Ia, Ib, IIa and IIb) in 55 wheat genotypes with 20 SSR primers. Plaschke et al. (1995) reported that the cluster analysis using 23 Wheat Microsatellite Consortium (WMC) SSR primers in 40 wheat cultivars and lines grouped data into one main cluster that comprised 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 19 sequence-related amplified polymorphic (SRAP) primers. Kumar et al. (2016) found that the cluster analysis broadly grouped 54 genotypes into four clusters 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. The cluster analysis, with the data of 22 EST-SSR, grouped the 64 durum wheat genotypes into 12 major groups. Thus, there appears to be some clustering based on the geographical origin of the genotypes, with some evidence for the improved varieties also being grouped together (Eujayl et al. 2002).

The PCoA and cluster analysis distinguished these cultivars into different groups (Gong et al. 2011). Nandha and Singh (2014) reported 16 EST-SSRs in 47 barley accessions (27 wild and 20 cultivated). A cluster analysis classified the cultivated and wild genotypes into two major groups corresponding to their geographic origin. The first principal coordinate accounted for 18.4% of the total variation, clearly separating the wild genotypes from the cultivated ones, and the second PCOA accounted for 10.4% of the total variation and comprised all cultivated genotypes. 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 breeding programs, wherein the genotypes with high genetic distance could be used.

Prasad et al. (2000) reported one to 13 alleles per locus in 55 wheat genotypes with 20 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 10 alleles per locus in 122 durum wheat genotypes, with 19 SSR markers. Al-Murish et al. (2013) reported a mean of 2.31 amplified bands (alleles) per marker among 17 coffee genotypes with 16 SSR markers and a mean PIC value of 0.43.

Eujayl et al. (2002) reported that a total of 89 alleles per locus ranged from one to seven alleles, with an average of 4.1 alleles per locus in 64 durum wheat genotypes, with 22 EST-SSR markers and a coefficient of similarity matrix ranging from 0.37 to 0.72.

The present analysis indicated that the polymorphism revealed by EST-SSR markers was lower than that by gSSR markers. It could effectively differentiate diverse genotypes. Similar results were observed by Eujayl et al. (2002), Xinquan et al. (2005), and Nandha and Singh (2014). The EST-SSR markers produced high-quality markers but had the minimum level of polymorphism (25%) in contrast to the other two exporters of genomic SSR primers (53%) (Eujayl et al. 2002). Recently, Wang et al. (2018) reported that the EST-SSR sequences were used to identify PIC and they were medium and ranged from 0.080 to 0.562, with a mean of 0.409, using 96 *Heterodera avenae* cysts with eight EST-SSR markers.

In the present study, five markers (barc63, barc124, barc125, barc144, and barc210) were amplified in the salt-tolerant genotypes. These markers could explain 65, 41, 63, 41, and 41%, respectively, of phenotypic variation. Similar results were reported for rice, where a good correlation was found between genetic diversity and phenotypic traits related to salt tolerance (Kordrostami et al. 2016). In addition, Hassanein and Al-Soqeer (2018) found a good correlation between genetic diversity and the morphological variability of *Moringa* genotypes.

The EST-SSR markers, Xcwem9 and Xcwem54, appeared to be linked to the salt tolerance genotypes. In addition, the specific DNA bands generated from EST-SSR markers could be used to differentiate between the salt-tolerant and salt-sensitive genotypes. Similar results were reported by Peng and Lapitan (2005) indicating the usefulness of EST-SSR markers (Xcwem9 and Xcwem54) for gene mapping and molecular breeding. In addition, Moghaieb et al. (2011) reported that the Egyptian wheat cultivars, 'Beni-Suef', 'Sohagby', and 'Gemmiza 10' were distinguished by SSR primers. Shahzad et al. (2012) found that 12 SSR markers (cfd 1, cfd 9, cfd 18, cfd 46, cfd 49, cfd 183, wmc 11, wmc 17, wmc 18, wmc 154, wmc 432, and wmc 503) detected specific alleles only in the salt-tolerant genotypes. Moreover, the SSR marker 'barc124' was associated with salt tolerance in wheat population (Shahzad et al. 2012).

Conclusions

In the present study, the wheat genotypes L2 and L4 exhibited the highest values for salt tolerance index. The SSR and EST-SSR primers (markers) were ideal for assessing the genetic variability in wheat. Moreover, five SSR markers (barc63, barc124, barc125, barc144, and barc210) were amplified in the salt-tolerant genotypes. The EST-SSR markers, Xcwem9 and Xcwem54, were identified in the salt-tolerant genotypes (L2, L4, and L6). Therefore, the EST-SSR and SSR markers were very useful in characterizing wheat genotypes that were mostly classified depending upon whether they were salt-tolerant or -sensitive.

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Author contributions

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