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

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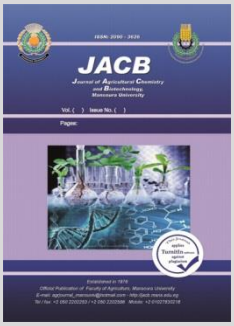

ABSTRACT

Generation mean analysis for salt tolerance in wheat was studied on P_1, P_2, F_1, F_2, BC_1 and BC_2 populations of three crosses. The cross-1 was between the salt tolerant Sakha-8 and sensitive Sham-8, cross-2 was between two salt tolerant varieties (Sakha-8 x Line-6) while cross-3 was between salt tolerant Line-6 and sensitive Sham-8. The genotypes were evaluated under control (0.0 mM NaCl) and salinity stress (150.0 mM NaCl) at germination and seedling stage for seven traits. The model of additive/dominance is inadequate for explaining the inheritance of all traits in control and salinity treatments in three crosses. The presence of epistasis in addition to the predominance of non-additive gene effects for all traits except SL indicated that the conventional selection procedure may not be effective enough to improve them. Therefore postponement of plant selection in later generations or the crosses between the selected segregants followed by selfing can be suggested to accumulate alleles favorable for the improvement of these traits. The molecular markers analysis revealed that only four SRAP primer combinations, three TRAP primer combinations and three SSR primer pairs generated polymorphic bands form the tested genotypes. The five polymorphic bands SRAP-2_{570bp}, SRAP-3_{760bp}, TRAP-1_{205bp}, TRAP-3_{450bp} and SSR-2_{215bp} appeared only in the tolerant genotypes. These markers may be considered as specific markers for salt tolerance. The identified markers in this study would allow implementation of marker-assisted selection to screen wheat segregating populations for salt tolerance.

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

INTRODUCTION

Salinity is the most important factor affected plant growth and decreased crop production in semi-arid and arid areas (Bai *et al.*, 2011). In Egypt, wheat is one of the most important and consumed food cereal, while its yield is seriously restricted by salinity and other abiotic stresses. One of the most important ways in Egypt to improve wheat yield is the producing stress-tolerant genotypes. The severity of salinity depends on the growth stage of wheat plants especially during germination, seedling growth, tillering, grain filling and finally reduce the biomass production and grain yield (Mass and Poss, 1989, Mirzaei *et al.*, 2012 and Öner and Kirli, 2018). Vigorous wheat seedlings lead to vigorous plants and good production under stress conditions. Screening wheat varieties for tolerance to salinity through germination of seeds and early seedling growth was carried out by Alom *et al.* (2016). Treatment with NaCl for 10 days affected wheat germination, shoot and root lengths as well as seedling dry wight. Significant positive correlations were found between salinity tolerance index and each of germination, shoot and root lengths, which reflects that these traits can be used to select and screen wheat varieties for salinity stress tolerance. The effect of salinity stress on wheat germination and seedling growth was studied by several workers (Ashraf *et al*., 1991; Raghav and Pal, 1994; Iqbal *et al.*, 1998; Mirzaei *et al.*, 2012 and Öner and Kirli, 2018).

Slow progress may be in improving the tolerance of salinity in wheat due to difficult examination of large numbers of accessions, varieties or plants in naturally saline soils due to temporal and spatial heterogeneity in the salinity of soil, lack of suitable selection trait which represents the tolerance of salinity and yield, and the in-sufficient knowledge about the genetic control and basis of tolerance to salinity (Akhtar *et al.*, 2012). One of the best strategies to reduce the effects of salinity in agriculture is to identify the mechanisms for salinity stress tolerance and breeding the new cultivars (Yamaguchi and Blumwald, 2005 and Forster *et al.*, 1990). In wheat, the genetic variation for tolerance to salinity, reliable method used for screening the genotypes for salt tolerance and a suitable breeding method are basic requirements for improving salt tolerance (Ali *et al.*, 2014 and Munns *et al.*, 2006).

Generation mean analysis (Mather and Jinks, 1982) is depends on the mean values of the six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2). Information resulted from this analysis can be utilized for the formulation of the effective strategy of breeding program. It explained the importance of additive and dominance gene effects in addition to the epistatic gene actions in determining the genotypic values of the individuals, families and generations. It is a simple analysis but useful method for determining gene effects in the polygenic quantitative traits, its greatest advantage lies in the ability to determine the epistatic gene effects including "dominance x dominance" interaction, "additive x additive" action, and "additive x dominance" interaction. Several

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researchers have been used generation mean analysis to study several quantitative traits in wheat including salt tolerance (Dashti *et al.*, 2010; Samineni *et al.*, 2011; Munns *et al.*, 2012 and Ali *et al.*, 2014). Ali *et al.* (2014) used generation mean effects to study salinity tolerance in seedling and adult stages of wheat plants.

One of the most important developments in the field of molecular biology is the use of molecular markers for the exploitation of DNA polymorphism in plants (Soto-Cerda and Cloutier, 2012). DNA markers provide a substantially unlimited number of molecular markers to compare the genotypes under different environmental conditions. They not associated with the stages of plant growth. DNA markers are located on the chromosomes and tightly linked to the gene(s) controlling the trait in the given population. Because salinity stress tolerance is polygenic trait and highly influenced by the genotype and environmental conditions, it is difficult to improve using the conventional breeding methods. Thus, the use of DNA markers are important in plant breeding, because of the absence of epistatic effects, the interaction between the genotypes and environment, in addition to ease of capture of homozygous plants that can be distinguished from other plants in the early generations (Kumar et al., 2015). DNA markers have been used to identify several QTLs or genes that contribute to salinity tolerance in cereals. The application of the indirect selection through DNA markers associated with the required trait is the well-known approach to improve crops having complex traits such as tolerance to salt stress (Im *et al.*, 2014).

Therefore, molecular markers including, target region amplification polymorphism (TRAP), sequence related amplified polymorphism (SRAP) as well as simple sequence repeat (SSR) (Islam, 2004; Al-Doss *et al.*, 2011; Ott *et al.*, 2011; Shahzad *et al.*, 2012; Barakat *et al.*, 2013; Miah *et al.*, 2013; Cui *et al.*, 2014; El-Rawyand Youssef, 2014; Shirnasabian *et al.*, 2014; Salem and Mattar, 2014; Kumar *et al.*, 2015; Abdelkhalik *et al.*, 2016) can play the important role to identify major genes controlling the salt tolerance, which will help plant breeders to select and identify the salt-tolerant genotypes that can be used as new source for future programs of crop breeding.

Bulk segregant analysis (BSA) is an efficient and rapid method used to identify molecular markers linked with the desired traits (Michemore *et al.*, 1991), the method depends on two bulked DNA of 10-12 plants selected from the two extremes of the segregated population. The two DNA bulks differed from each other in the trait of interest (i.e tolerant vs. sensitive) and the genome regions associated with such trait should be enriched, while other regions of the genome are assumed to be similar between the two bulks. The two bulks are screened and genotyped with sufficient markers to cover the full genome. (Ford *et al.*, 1996).

Thus, the objectives of the present study were to determine gene actions and inheritance pattern of salt tolerance at germination and seedling stage in bread wheat using generation mean analysis, in order to identify the most effective criteria and proper breeding strategy for salinity tolerance in wheat. In addition to validate these screening criteria using different molecular markers linked to salt tolerance in wheat.

MATERIALS AND METHODS

The experimental material consisted of the six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) derived from three wheat crosses. The First cross was between the local cultivar sakha-8 and sham-8, second cross was between two local varieties (Sakha-8 x Line-6) while the third cross was between Line-6 and Sham-8. Both sakha-8 and Line-6 characterized as salinity tolerant while sham-8 was sensitive to salinity stress (Table 1).

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

Cross	Parental name	Pedigree	Drought	Origin
Cross-1	SAKHA8	CNO67//SN64/KLRE/3/8156	tolerance	Egypt
	SHAM-8	ICARDA	susceptible	ICARDA
Cross-2	SAKHA8	CNO67//SN64/KLRE/3/8156	tolerance	Egypt
	Line-6	*Selected for heat tolerance (G-164 x US3)*	tolerance	Egypt
	SHAM-8	ICARDA	susceptible	ICARDA
Cross-3	Line-6	*Selected for heat tolerance (G-164 x US3)*	tolerance	Egypt

The study was carried out at Genetics department and the experimental farm of Faculty of Agriculture, Assiut University, Egypt, during the period from 2015-2019. In 2015/2016 season, the three crosses were made among the parents to produce F_1 hybrid seeds. In 2016/2017 season, the F_1 plants were selfed to produce F_2 seeds and backcrossed to the parents to produce BC_1 and BC_2 seeds. The six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of the three crosses were used to study the inheritance and genetic components of salinity tolerance at germination and seedling stage in a laboratory experiment. Wheat grains of each genotype were disinfected by immersion in 30% Clorox (7% sodium hypochlorite) for 5 min, then rinsed three times with distilled water and allowed to germinate in aluminum trays (25cm wide × 50cm long × 6cm deep), which were filled with sterilized sand. From a preliminary experiment, it was found that the 150 mM NaCl was suitable for salinity evaluation and selection in the tested genotypes (un-published data). Salinity stress was simulated by irrigation with NaCl solution at concentrations of 0.0 and 150mM (w/v). Distilled water was used for the control treatment. The experiment was carried out in a randomized complete block design with three replicates. 20 grains from each parent and F_1, 30 grains from each F_2, BC_1 and BC_2 populations were planted in each replicate. The aluminum trays were covered with transparent plastic sheets and incubated under laboratory condition (24±2 ^{o}C) for 14 days. The percentage of seed germination (GP%), root length (cm), shoot length (cm), root fresh weight (mg), shoot fresh weight (mg), root dry weight (mg) and shoot dry weight (mg) were measured on 15 plants from each parent and F_1, 30 plants from each F_2, BC_1 and BC_2 populations in each replicate. Dry weight (mg) was measured after drying samples at 70 °C for 72 h in an oven.

Data analysis:

Data obtained from the studied traits were subjected for analysis of variance as portrayed by Steel *et al.* (1997) to find variations between all six generations of both cross combinations.

Salinity stress susceptibility index (SSI) was calculated as for each genotype using the following equation according to (Fischer and Maurer, 1978).

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

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

SI that is stress intensity, where:

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

The scaling tests (A, B, C and D) were carried out to identify involvement of epistasis as indicated by Mather and Jinks (1982). If epistasis was present, analysis for estimation of non-allelic interaction was done for estimation of six parametric models of inheritance viz., the mean of all generation [m], additive effects [a], dominance effects [h], additive x additive interaction [i], additive x dominance [j] and dominance x dominance [l] as mentioned by Hayman (1958).

Molecular markers:

This part of this study was carried out in Biotechnology laboratory at Genetics department and the experimental farm of Faculty of Agriculture, Assiut University, Egypt.

Isolation of genomic DNA

Total genomic DNA was isolated from young leaves of wheat plants using CTAB protocol with some modifications (Murray and Thompson 1980). RNA was removed from the DNA preparation by applying 10.0 µl of RNAase (10.0mg/ml) and incubated for 30 minutes at 37°C. The concentration of DNA samples were quantified by using a spectrophotometer.

Bulked segregant analysis (BSA)

DNA isolated from P_1, P_2, F_1, tolerant and sensitive plants from each of BC_1 and BC_2 populations (10 tolerant plants and 10 sensitive plants) based on data recorded on seed germination and seedling growth traits of three crosses, Sakha-8 (P_1) x Sham-8 (P_2), Sakha-8 (P_1) x Line-6 (P_2) and Line-6 (P_1) x Sham-8 (P_2) were subjected to bulk segregant analysis (BSA) (Quarrie *et al.* 1999) with sequence-related amplified polymorphism (SRAP), target region amplification polymorphism (TRAP) and simple sequence repeat (SSR) markers to identification of marker closely linked to salt tolerant.

Five sequence related amplified polymorphism (SRAP) primer combinations, five target region amplification polymorphism (TRAP) combinations and five simple sequence repeat (SSR) microsatellite primer pairs (Table 2), obtained from Metabion International AG Company (Germany), were tested in this investigation to amplify the template DNA. Amplification reactions were performed in 25 µL volumes, containing 3.0 µL reaction buffer (10×), 11.7 µL dH_2O, 3.0 µL dNTP's mix (2.5 mM each dNTP; Promega), 1.0 µL reverse primer, 1.0 µL forward primer (2.5 µM) for each SRAP, TRAP and SSR markers, 0.25 µL Taq DNA polymerase (5 U/µL; Promega), 4.0 µL $MgCl_2$ (25 mM), and 1 µL template DNA (50 ng/µL). Amplification conditions were performed in a Lab thermal Cycler (Model SensoQuest, GmbH, Göttingen, Germany) with the following specification: For SRAP and TRAP markers: 5 min at 94 °C followed by 10 cycles: 1 min at 94 °C, 1 min at 35 °C and 2 min at 72 °C then 35 cycles with 1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C then finally extension for 10 min at 72 °C. For SSR markers: 5 min denaturation at 94 °C, then 45 cycles comprising 94 °C for 60 s, annealing of primer for 60 s at 58–60 °C, extension for 60 s at 72 °C followed by final extension for 10 min at 72 °C. The PCR products were separated in an Ultra-Pure agarose gel with 2.5 % concentration, at 80 V for 3–3.5 h. Gels were stained with ethidium bromide (EB) (0.5 µg/mL) and DNA fragments were visualized using GelDoc-It®^{2} Imager. The obtained data of SRAP, TRAP and SSR markers were scored on the basis of presence (1) or absence (0) of a given marker, after excluding unreproducible bands.

Table 2. Code and sequence of primers that gave polymorphism.

Primer codes		Sequence (5' to 3')
		TRAP primer
F	TRAP-1	5′–TGAGTCCAAACCGGAAT–3′
R		5′–TCACCCGCACCTTCTTCC–3′
F	TRAP-2	5′–TGAGTCCAA ACCGGAGC–3′
R		5′–TCACCCGCACCTTCTTCC–3′
F	TRAP-3	5′–GAGTCCAAACCGGAGC–3′
R		5′–CCC TCCACCAATCACAAT–3′
		SRAP Primer
Me-1	SRAP-1	5′–TGAGTCCAAACCGGATA–3′
Em-3		5′–GACTGCGTACGAATTGAC–3′
Me-3	SRAP-2	5′–TGAGTCCAAACCGGAAT–3′
Em-3		5′–GACTGCGTACGAATTGAC–3′
Me-5	SRAP-3	5′–TGAGTCCAAACCGGAAG–3′
Em-5		5′–GACTGCGTACGAATTAAC –3′
Me-4	SRAP-4	5′–TGAGTCCAAACCGGACC–3′
Em-5		5′–GACTGCGTACGAATTAAC–3′
		SSR Primer
F	Xtxp-8	5′–ACAT CTACT AC CCT CTCACC–3′
R	(SSR-1)	5′–ACACATCGAGACCAGTTG–3′
F	Xtxp-12	5′–ATAT GGAAGGAAGAAGC C GG–3′
R	(SSR-2)	5′–AACACAACAT GCAC GCAT G–3′
F	Xtxp-19	5′–ATACTATCAAGAGGGGAGC–3′
R	(SSR-3)	5′–AGTACTAGCCACACGTCAC –3′

RESULTS AND DISCUSSION

The severity of salinity depends on the growth stage of wheat plants especially during germination, seedling growth, tillering, grain filling and finally reduce the biomass production and grain yield (Mirzaei *et al.*, 2012). Vigorous wheat seedlings lead to healthy plants and ultimately good production under stress conditions. Therefore, the germination of seeds, seedling vigor and shoot and root lengths are prerequisites for the establishment of wheat under salinity conditions (Öner and Kirli, 2018). In the present study, the performance of wheat P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of cross-1 (Sakha-8 x Sham-8), cross-2 (Sakha-8 x Line-6) and cross-3 (Line-6 x Sham-8) at germination and early seedling growth stage was studied under salinity stress (150 mM NaCl) as compared with the non-stressed control treatment.

The mean values of germination percentage (GP), shoot length (SL), root length (RL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) in P_1, P_2, F_1, F_2, BC_1 and BC_2 populations of the three crosses under control and salinity stress are given in Table (3). The analyses of variance (Table 4) revealed significant differences between the studied generations in all traits under study as well as between

control and salinity treatments in the three crosses, indicating the existence of genetic variations and possibility of selection for salinity tolerance. The "genotypes x salinity treatments" interaction was also significant in all traits in the two crosses, except for RFW, SFW in cross-1 and RDW in cross-2, displaying their variable responses from one treatment to another, and similar responses in the respective RFW, SFW and RDW.

Table 3. The mean values of germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg), Shoot dry weight (SDW, mg) and Salinity Susceptibility Index (SSI) in six populations of cross-1 (sakha-8 x sham-8), cross-2 (sakha-8 x Line-6) and cross-3 (Line-6 x Sham-8) on control (C) and salinity (S) stress (150 mM NaCl).

Populations	GP		RL		SL		RFW		RDW		SFW		SDW		SSI
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	
Cross-1 (sakha-8 x sham-8)															
P_1	98.33	65.00	14.62	4.73	15.98	7.45	91.67	44.00	30.00	9.00	110.33	66.33	34.00	12.67	0.87
P_2	96.67	48.33	12.91	2.67	13.47	3.92	71.67	28.33	22.33	4.33	80.0	55.33	13.33	9.33	1.12
F_1	100.0	61.67	14.5	3.73	16.76	4.43	86.33	43.33	34.33	16.67	106.0	64.00	31.33	13.67	0.89
F_2	96.67	51.67	13.47	3.14	16.3	4.49	83.00	32.67	29.00	4.67	49.33	47.67	28.33	12.67	1.04
Bc_1	96.67	48.33	13.6	3.17	14.62	6.27	85.00	30.00	28.33	4.00	95.33	42.00	24.33	12.33	0.95
Bc_2	96.67	36.67	12.30	2.45	12.91	3.14	65.33	19.67	19.00	2.67	89.00	31.67	20.67	10±0	1.16
L.S.D 0.05	4.98	8.73	0.30	0.22	0.16	0.46	7.60	8.79	5.61	2.57	60.41	5.47	2.88	3.30	
Cross-2 (sakha-8 x Line-6)															
P_1	100.0	65.00	14.30	4.73	15.98	7.45	90.00	44.00	14.33	9.00	110.33	60.67	34.00	17.00	0.87
P_2	98.33	50.00	13.93	3.16	15.17	4.54	78.00	31.00	11.00	7.33	103.33	47.00	16.67	9.33	0.96
F_1	100.0	60.00	12.00	4.49	17.60	6.9	86.33	45.33	13.33	7.67	74.33	51.33	15.00	14.17	0.91
F_2	100.0	50.00	11.29	3.92	16.83	6.27	71.00	28.33	11.67	6.67	67.33	47.33	10.33	8.67	1.14
Bc_1	100.0	60.00	14.17	4.43	14.30	7.03	87.00	35.00	13.67	7.33	109.0	59.00	32.00	19.33	0.95
Bc_2	100.0	48.33	12.93	3.14	13.93	4.27	73.33	31.33	9.33	7.00	70.67	46.33	14.00	8.33	1.20
L.S.D 0.05	2.14	10.72	0.20	0.45	0.24	0.37	6.89	6.47	4.01	1.76	14.70	9.95	9.26	9.53	
Cross-3 (Line-6 x Sham-8)															
P_1	100	65.00	14.00	4.73	15.17	7.45	78.00	44.00	11.33	9.00	103.3	60.67	16.67	17.00	0.88
P_2	93.33	50.00	11.91	3.16	13.47	4.54	71.67	31.00	10.33	7.33	80.00	47.00	13.33	9.33	1.14
F_1	96.67	60.00	12.27	4.49	17.68	6.90	71.67	45.33	11.5	7.67	75.00	51.33	15.00	14.17	0.86
F_2	95.2	50.00	9.93	3.92	17.33	6.27	69.00	28.33	8.33	6.67	72.5	47.33	8.33	8.67	1.07
Bc_1	96.67	60.00	9.29	4.43	14.00	7.03	73.33	35.00	8.07	7.33	78.00	59.00	17.67	19.33	0.90
Bc_2	95.00	48.33	8.82	3.14	11.91	4.27	65.33	31.33	6.03	7.00	70.00	46.33	14.00	8.33	0.88
L.S.D 0.05	4.60	10.72	1.25	0.45	0.28	0.37	9.87	6.47	3.39	1.76	15.25	9.95	8.26	3.53	1.14

Table 4. The analyses of variance for germination percentage (GP), shoot length (SL), root length (RL), Root fresh weight (RFW), Root dry weight (RDW), Shoot fresh weight (SFW) and Shoot dry weight (SDW) in six populations of cross-1 (sakha-8 x sham-8), cross-2 (sakha-8 x Line-6) and cross-3 (Line-6 x Sham-8) on control and salinity stress.

Source	DF	Mean of Squares						
		GP	RL	SL	RFW	RDW	SFW	SDW
Cross-1 (sakha-8 x sham-8)								
Replicates	2	4.86	0.021	0.006	13.08	29.36	462.25	0.194
Genotypes (G)	5	192.78**	4.28**	11.12**	526.25**	118.43**	793*	179.04**
Salinity (S)	1	18677.78**	945.66**	910.13**	20300.25**	3700.69**	12432.25**	940.44**
G. X S	5	127.78**	0.179**	4.15**	31.58	56.69**	1201.71	129.84**
Error	22	13.95	0.019	0.04	19.69	119.97	547.64	2.65
Cross-2 (sakha-8 x Line-6)								
Replicates	2	52.77	0.01	0.02	34.19	1.69	0.11	28.32
Genotypes (G)	5	79.02**	3.28**	8.74**	160.71**	9.49*	908.78**	230.31**
Salinity (S)	1	17556.25**	749.27**	822.41**	18315.11**	200.69**	12469.44**	510.01**
G. X S	5	67.91*	2.83**	2.93**	181.71**	3.23	461.91**	139.37**
Error	22	19.44	0.04	0.03	12.49	2.76	47.89	13.55
Cross-3 (Line-6 x Sham-8)								
Replicates	2	0.006	3.61	0.22	0.012	0.002	0.11	0.002
Genotypes (G)	5	0.09**	16.58**	2.54**	0.82**	0.015**	0.45**	0.018**
Salinity (S)	1	1.43**	238.8**	980.15**	0.915**	0.03**	5.47**	0.08**
G. X S	5	0.07**	11.34**	4.76**	0.008*	0.002**	0.087**	0.002**
Error	22	0.002	2.38	0.74	0.05	0.002	0.034	0.0004

The results also revealed that salinity stress significantly decreased the performance of all studied traits of all wheat genotypes in comparison to the control treatment. The reduction in germination rate and percentage of wheat genotypes with salinity stress was also observed by Ashraf *et al*. (1991) and Raghav and Pal (1994). Highly significant decrease in root length, shoot length and dry weights as the salinity levels increased were recorded in wheat cultivars by Iqbal *et al.* (1998) and Mirzaei *et al.* (2012). Moreover, Öner and Kirli (2018) reported that germination parameters of wheat cultivars negatively influenced by salt doses greater than 25 mM. Dry coleoptile and radicula weights and lengths decreased with increasing salt concentrations. They concluded that salinity negatively influenced germination times and seedling growth of bread wheat cultivars.

Lowest values of salinity susceptibility index (SSI) were observed for P_1 (0.87), F_1 (0.89) and BC_1 (0.95) in cross-1, and for P_1 (0.87) followed by the F_1 (0.91), BC_1 (0.95) and P_2 (0.96) populations in cross-2 and for F_1 (0.86) followed by the P_1 (0.88), BC_2 (0.95) and BC_2 (0.96) populations in cross-3. These result indicated that selection in the segregation population for salinity tolerance could be effective to produce lines have high tolerance to salinity stress at germination and early stage of seedling development.

Scaling tests

Choice the efficient breeding programs depends on the knowledge of the genetic system controlled the trait to be selected, the estimates of different types of gene effects contributing to the genetic variability are shown in Tables (5-8). The presence or absence of non-allelic gene interaction or epistasis can be detected by generation means analysis using the scaling test (Sharmila *et al.,* 2007). The results of A, B and C scaling test for cross-1 (Sakha-8 x Sham-8), cross-2 (Sakha-8 x Line-6) and cross-3 (Line-6 x Sham-8) under control and salinity stress, revealed that the significance of any one of the A, B and C parameters indicates the presence of epistatic effects on the scale of measurement used.

Table (5) of scaling test showed that additive–dominance model is inadequate for explaining the inheritance of all traits of the three crosses in control and salinity treatments, indicating the presence of non-allelic gene interaction for these traits.

Table 5. Scaling test for germination percentage (GP), shoot length (SL), root length (RL), Root fresh weight (RFW), Root dry weight (RDW), Shoot fresh weight (SFW) and Shoot dry weight (SDW) in six populations of cross-1 (sakha-8 x sham-8), Cross-2 (sakha-8 x Line-6) and cross-3 (Line-6 x Sham-8) on control and salinity stress.

Traits	Control			Salinity stress		
	A	B	C	A	B	C
	cross-1 (sakha-8 x sham-8)					
GP	-0.16**	-0.2**	-0.23**	-0.04*	-0.6**	-0.25**
RL	-4.42**	-7.86**	8.84**	1.73**	1.25**	3.03**
SL	-3.03**	-7.07**	-0.09	-2.82**	-0.48**	-11.36**
RFW	0.31*	-0.11	1.83**	0.01	-0.07	3.91**
RDW	0.3*	0.001	0.33**	-0.05*	-0.03**	0.73**
SFW	0.62**	0.51*	2.47**	-0.21	-0.25*	0.83**
SDW	0.09**	0.06**	0.60**	-0.05*	-0.08**	0.60**
	Cross-2 (sakha-8 x Line-6)					
GP	0.063**	-0.26**	-0.14**	0.19**	0.17**	0.52**
RL	-4.17**	-3.98**	6.06**	2.24**	2.39**	7.87**
SL	2.03**	0.72	2.67**	6.6**	3.36**	11.08**
RFW	0.26**	-0.22**	-0.22**	0.14*	0.04	4.72**
RDW	-0.08**	-0.06*	0.29**	0.02	-0.05**	0.67**
SFW	0.59**	0.34*	5.51**	0.39**	0.11	4.35**
SDW	0.06*	0.04	0.89*	0.04	-0.02	0.5**
	Cross-3 (Line-6 x Sham-8)					
GP	-0.34**	-0.51**	0.04	-0.43**	-0.56**	-0.99**
RL	-3.44**	-1.85**	21.23**	2.43**	0.39	5.14**
SL	1.05**	0.10	-2.60**	2.91**	-3.23**	5.02**
RFW	-0.09*	-0.28**	3.62**	-0.21**	-0.28**	3.04**
RDW	0.06**	-0.02	0.30**	-0.06**	-0.05**	0.50**
SFW	0.70**	0.60**	3.77**	-0.22**	-0.24**	1.86**
SDW	0.06**	0.01	0.50**	-0.08**	-0.11**	0.40**

In control treatment, the three types of scaling test (A, B and C) were important for GP, SFW, SDW and RL in coross-1, and for all traits, except for SL and SDR in cross-2, and for RL, RFW and SFW in cross-3. Under salinity stress, the three types were significant for all traits except SFW and RFW in cross-1, and for GP, SL and RL in cross-2, and for all traits except for RL in cross-3. Under control treatment, the A and B parameters were significant for SL in cross-1 and for GP in cross-3 while, the A and C types were important for RFW and RDW in cross-1, for SL and SDW in cross-2 and for SL, RDW and SDW in cross-3. Under salinity stress, the A and C types were important for RFW and SFW in cross-2 and for RL only in cross-3. Also under salinity stress, both B and C types of scaling test were important for SFW in cross-1 and for RDW in cross-2. While, the C parameter was important for RFW in cross-1 and SDW in cross-2. These results may be taken as an evidence for the failure of simple genetic model to ascertain the genetic variation for these characters in the corresponding crosses. Therefore, the six parameters model was applied for these characters in order to assess the digenic interaction types controlling the genetic variations (Mather and Jinks, 1982).

Types of gene effects and components of variances

Values of the mean parameter (m) were significant for all studied traits of the three crosses in both treatments indicating the contribution of overall mean plus the locus effects and interaction of the fixed loci (Tables 6, 7 and 8).

Additive gene action is the most important genetic parameter for selection of the desired trait in breeding program since they are stable fixable component of polygenic variability (Kearsey and Pooni, 1998). In cross-1, the additive gene effects [d] was significant for RL and SL in the control treatment and for GP, SL, RFW, RDW and SDW under salinity stress. In cross-2, additive gene effects [d] was significant for GP and RFW under control treatment and for all traits except SFW under salinity stress treatment. In cross-3, additive gene effects [d] was significant for all traits except for GP and RL under control treatment and RDW under salinity stress treatment. These results indicating the importance additive gene effects in the inheritance of these traits and the potentiality of improving their performance using the pedigree selection program may be more effective.

The estimates of dominance gene action [h] were significant for all traits under both treatments in the three crosses, except RL in cross-1 under salinity stress and SL in cross-2 under control and salinity stress. These results indicating the importance of dominance gene effects in the inheritance of these traits. The magnitudes of dominance effect [h] were higher than additive effect [d] in all traits indicating that these traits mainly controlled by dominance gene action, while additive gene effects were also important.

Estimates of epistatic gene effects:

Estimates of non-allelic gene interactions were also determined and presented in Tables (6, 7 and 8). In cross-1, the three types of non-allelic gene interactions (i, j, l) were significant for SL and RL in the control treatment and for GP, SL and SDW under salinity stress. While in cross-2, the [i], [j] and [l] were significant for RFW in the control, and for SDW and RDW under salinity stress while, in cross-3 the [i], [j] and [l] were significant for all traits except GP and SFW under the control treatment whereas, under salinity stress these parameters were significant for SL, RFW and SDW.

The additive x additive [i] and dominance x dominance [1] effects in cross-1 were important for RFW and RDW in both treatment, GP and SDW in the control and SFW under salinity stress. In cross-2, the [i] and [1] gene interactions were important for RL, RDW, SFW and SDW in the control treatment, and for SFW and RFW under salinity stress. In cross-3, the [i] and [1] parameters were important for SFW under both treatments and for GP and RDW under control and salinity stress, respectively.

Table 6. Additive-dominance analysis for germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg) and Shoot dry weight (SDW, mg) in six basics for cross-1 (sakha-8 x sham-8) on control and salinity stress (15، mM NaCl).

Gene effects	GP	RL	SL	RFW	RDW	SFW	SDW
				Control			
M	96.67**±1.67	13.47**±0.03	16.3**±0.06	83**±1.53	29**±3.46	49.33**±2.19	8.33**±0.33
[d]	0.05±0.02	2.11**±0.5	1.92**±0.45	0.03±0.07	0.02±0.02	0.07±0.12	0.02±0.01
[h]	-0.18**±0.06	-25.7**±3.09	-10.71**±1.6	-3**±0.47	-0.42**±0.07	-1.7**±0.51	-0.55**±0.04
[i]	-0.13*±0.06	-21.12**±2.93	-10.01**±1.4	-2.25**±0.46	-0.33**±0.06	-1.33**±0.5	-0.45**±0.04
[j]	0.022±0.02	1.72*±0.78	2.02**±0.54	-0.1±0.12	-0.002±0.02	0.05±0.14	0.012±0.01
[l]	0.5**±0.1	33.41**±3.92	20.11**±2.61	2.68**±0.56	0.32**±0.09	0.2±0.69	0.3**±0.06
				Salinity stress			
M	51.67**±4.41	3.14**±0.03	4.49**±0.6	32.67**±1.45	4.67**±0.33	47.67**±3.18	12.67**±0.67
[d]	0.31**±0.01	0.28±0.18	1.31**±0.07	0.08**±0.03	0.02*±0.01	0.05±0.05	0.02*±0.01
[h]	-0.72**±0.02	-0.31±0.51	8.27**±0.26	-4.18**±0.18	-0.86**±0.07	-1.38**±0.15	-0.79**±0.03
[i]	-0.4**±0.02	-0.05±0.48	8.06**±0.23	-3.97**±0.18	-0.82**±0.07	-1.27**±0.13	-0.73**±0.03
[j]	0.28**±0.01	0.24±0.21	-1.17**±0.09	0.04±0.03	-0.01±0.01	0.03±0.07	0.02*±0.01
[l]	1.04**±0.04	-2.94**±0.87	-4.75**±0.42	4.03**±0.21	0.9**±0.07	1.72**±0.27	0.86**±0.05

Table 7. additive dominance analysis for germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg) and Shoot dry weight (SDW, mg) in six basics for cross-2 (sakha-8 x Line-6) on salinity stress (15، mM NaCl).

Gene effects	GP	RL	SL	RFW	RDW	SFW	SDW
				Control			
M	100**±0.00	11.29**±0.12	16.83**±0.06	71**±2.08	11.67**±0.88	67.33**±2.33	10.33**±1.45
[d]	0.16**±0.01	0.37±0.63	0.7±0.37	0.07**±0.01	0.01±0.01	0.07±0.08	0.01±0.01
[h]	-0.21**±0.04	-19.82**±1.68	-0.68±0.82	-3.65**±0.22	-0.58*±0.03	-5.5**±0.49	-0.93**±0.05
[i]	-0.05±0.04	-14.16**±1.62	0.08±0.76	-3.14**±0.22	-0.43**±0.02	-4.58**±0.49	-0.79**±0.05
[j]	0.16**±0.01	-0.07±0.66	0.66±0.37	0.24**±0.02	-0.01±0.02	0.12±0.11	0.01±0.02
[l]	0.25**±0.06	22.26**±2.89	-2.82±1.61	3.1**±0.23	0.56**±0.05	3.65**±0.58	0.69**±0.07
				Salinity stress			
M	65**±2.89	4.73**±0.03	7.45**±0.05	44**±2.08	9**±0.58	60.67**±2.33	17**±0.58
[d]	0.1**±0.01	0.64**±0.24	2.05*±0.93	0.087*±0.04	0.05**±0.01	0.14±0.08	0.04**±0.01
[h]	-0.27**±0.07	-3.54**±0.67	-0.67±2.73	-4.74**±0.57	-0.74**±0.08	-3.91**±0.72	-0.495**±0.07
[i]	-0.16**±0.06	-3.24**±0.67	-1.12±2.69	-4.55**±0.56	-0.69**±0.08	-3.85**±0.72	-0.48**±0.07
[j]	0.01±0.02	-0.07±0.24	1.62±0.94	0.05±0.04	0.035**±0.01	0.14±0.09	0.03*±0.02
[l]	-0.2±0.11	-1.38±1.08	-8.84*±4.31	4.37**±0.58	0.72**±0.09	3.35**±0.78	0.46**±0.09

Table 8. additive dominance analysis for germination percentage (GP%), shoot length (SL, cm), root length (RL, cm), Root fresh weight (RFW, mg), Root dry weight (RDW, mg), Shoot fresh weight (SFW, mg) and Shoot dry weight (SDW, mg) in six basics for cross-3 (Line-6 x Sham-8) on salinity stress (15، mM NaCl).

Gene effects	GP	RL	SL	RFW	RDW	SFW	SDW
				Control			
M	95**±2.89	9.93**±0.07	17.33**±0.23	69**±4.04	8.33**±0.33	72.5**±7.22	8.33**±0.33
[d]	0.1±0.07	0.46±0.27	1.36**±0.12	0.13**±0.01	0.05**±0.01	0.09*±0.04	0.04**±0.01
[h]	-0.93**±0.24	-30.77**±2.63	4.58**±0.68	-4.34**±0.15	-0.35**±0.04	-2.73**±0.11	-0.52**±0.03
[i]	-0.89**±0.23	-26.52**±2.6	3.75**±0.66	-4.00**±0.14	-0.25**±0.04	-2.47**±0.09	-0.43**±0.03
[j]	0.082±0.08	-0.797**±0.3	0.48**±0.18	0.095**±0.03	0.04**±0.02	0.05±0.06	0.025**±0.01
[l]	1.74**±0.36	31.81**±2.88	-4.9**±0.83	4.38**±0.16	0.21**±0.06	1.18**±0.2	0.36**±0.04
				Salinity stress			
M	50**±2.89	3.92**±0.02	6.27**±0.06	28.33**±1.67	6.67**±0.33	47.33**±2.6	8.67**±0.33
[d]	0.11**±0.01	0.91**±0.16	2.33**±0.27	0.05**±0.003	0.01±0.004	0.03*±0.01	0.03**±0.003
[h]	-0.41**±0.05	-2.91**±0.64	-7.69**±0.7	-3.73**±0.31	-0.66**±0.03	-2.46**±0.2	-0.63**±0.02
[i]	0.001±0.05	-2.33**±0.64	-5.34**±0.68	-3.52**±0.31	-0.61**±0.03	-2.31**±0.2	-0.59**±0.02
[j]	0.06**±0.01	1.02**±0.17	3.07**±0.31	0.03**±0.01	-0.004±0.004	0.01±0.01	0.02**±0.003
[l]	0.99**±0.06	-0.49±0.85	5.66**±1.22	4.01**±0.31	0.71**±0.03	2.77**±0.21	0.78**±0.02

In cross-1, the additive x additive interaction [i] was significant in SFW in the control treatment while dominance x dominance [1] was important for RL under salinity stress.

In GP of cross-2, the additive x dominance [j] and dominance x dominance [1] were important in the control treatment while additive x additive [i] is important under salinity stress. Also under salinity stress, cross-2 showed the importance of additive x additive in RL while, dominance x dominance action [1] was important for SL.

In cross-3, the additive x additive [i] and dominance x dominance [l] effects were important for SFW under both control treatments while, these parameter were important for

GP under control treatment and for RDW under salinity stress. In GP of cross-3, the additive x dominance [j] and dominance x dominance [1] were important in the salinity stress treatment.

The significance of both additive x additive [i] and dominance x dominance [l] effects increase the variation between the generation and in the segregating population (Said, 2014).

Negative [h] and [i] for all traits, except SL under salinity stress in cross-1, showed involvement of decreasing alleles of dominant and additive × additive epistatic genes from susceptible parent (sham-8) in the character expression.

In the present study, the presence of epistasis in addition to the predominance of non-additive gene effects for all traits except SL indicating that conventional selection procedure may not be effective enough to improve them. Therefore it may be suggested that the postponement of selection to the subsequent later generations or hybridization between the selected plants followed by one or two selfing generations allow the accumulation of favorable alleles for the improvement of these traits. Similar conclusion was also reached by (Sharmila *et al.,* 2007; Iqbal *et al.,* 2012; Khan *et al.,* 2016; Ahmed *et al.*, 2019).

Positive [d] and [l] in all traits, except SL and RL under stress, suggests that additive effects and "dominance X dominance" interaction are responsible for the increase in these traits under salinity stress treatment. Meanwhile, the involvement of duplicate gene action in the inheritance of all traits under both treatments offered a complex situation and suggested delaying the plant selections to later generations. Similar results were also obtained by (Khan *et al.*, 2016).

The significance of the [i], [j] and [l] estimates and their magnitudes indicated that epistatic gene effects are important in the basic mechanism of seedling traits contributed in salinity tolerance inheritance in the studied wheat crosses. Hayman (1960) reported that when epistasis is of major importance in inheriting the trait, it is difficult to obtain unbiased estimates of pooled dominance or additive effects. Also, regarding the presence of additive/dominance effects in the genetic control of the traits in these crosses, the recurrent selection and then followed by pedigree breeding or by the selective mating system may be useful to improve salinity tolerance in wheat (Dehdari *et al.,* 2007).

From the above results we may conclude that the estimated types of gene effects provided a test for gene action and are useful to analyze the genetic profiles of wheat genotypes so as to improve the desirable traits. The estimates of gene actions obtained from each cross may be specific to that cross and not be applicable to the other crosses. Hybridization and adoption of recombinant breeding strategy could be the way forward for developing salinity-tolerant genotypes from the crosses (sakha-8 x sham-8), (sakha-8 x Line-6) and (Line-6 x Sham-8).

Molecular Markers

Molecular polymorphism among three parents Sakha-8 (tolerant), Sham-8 (sensitive) and Line-6 (tolerant) was assessed using three molecular marker systems (SRAP, TRAP and SSR) with 15 primers. Out of 15 primer pairs, 10 (66.67%) (4 SRAP, 3 TRAP and 3 SSR) were polymorphic among the parental genotypes and were used for further BSA analysis.

In this study, the monomorphic primers were excluded from the analysis. A total of 27 bands were generated by the SRAP primers, and out of these, 13 (48.15%) were polymorphic with an average of 3.25 polymorphic bands per primer pairs (Fig. 1). Out of 13 polymorphic bands, 3 positive bands were unique for Sakha-8 (tolerant), 6 (one positive and 5 negative) were unique for Sham-8 (sensitive) and 4 positive band were unique for Line-6 (tolerant) (Fig. 1 and Table 9).

A total of 32 TRAP bands were produced by TRAP primer combinations, 19 (59.38%) out of them were polymorphic (Fig. 2). TRAP-3 gave the heist number of polymorphic bands (10), followed by TRAP-1 (6 bands) while TRAP-2 gave the lowest number (3 bands) with an average of 6.33 bands per primer combination. Out of 19 polymorphic bands, 5 were specific for Sakha-8 (4 positive and 1 negative), 10 bands were specific for Sham-8 (4 positive and 6 negative) and 4 were specific for Line-6 (3 positive and 1 negative) (Fig. 2 and Table 9).

SSR primer pairs amplified a total of 24 alleles, out of them 15 (62.5%) showed polymorphism with an average of 5 polymorphic alleles per primer pair (Fig. 3). The number of alleles per locus were 8 (SSR-1), 9 (SSR-2) and 7 (SSR-3) with an average of 8 alleles per primer pairs. The heist number of specific bands (8) was recorded for Sham-8 (3 positive and 5 negative) followed by Line-6 (5 bands, 3 positive and 2 negative) while the lowest number was recorded for Sakha-8 (2 bands, 1 positive and 1 negative) (Fig. 3 and Table 9).

These results are in agreement with Bibi *et al.* (2009); Ojaghi and Akhundova (2010); Salem and Mattar (2014); Shahzad *et al.* (2012), Ahmad *et al.* (2013), Khaled and Hamam (2015), Abdelkhalik *et al.* (2016), Kumar *et al.* (2016) and Salehi *et al.*, (2018) who reported high molecular genetic variation in wheat genotypes.

Table 9. Positive and negative unique markers for three parents generated by SRAP, TRAP and SSR markers.

	Shka-8		Sham-8		Line-6	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
SRAP-1	---	---	---	---	1055bp	---
SRAP-2	-----	---	1350bp	570bp, 255 bp	865bp	---
SRAP-3	1385bp, 630bp	---	---	760bp	---	---
SRAP-4	535bp	---	---	340bp, 220bp	450bp, 280bp	---
TRAP-1	655bp, 185bp		475 bp	205bp	350bp, 245bp	---
TRAP-2	255bp	75bp	90bp	---	---	---
TRAP-3	695bp	---	790bp, 575bp	650bp, 635bp, 545bp, 450bp, 715bp	350bp	835bp
SSR-1	320bp	---	680bp	1345bp, 245bp	1130bp	---
SSR-2	---	755bp	815bp, 510bp	215bp	650bp, 555bp	---
SSR-3	---	---	---	455bp, 225bp		380bp, 185bp
Total	8	2	8	16	10	3

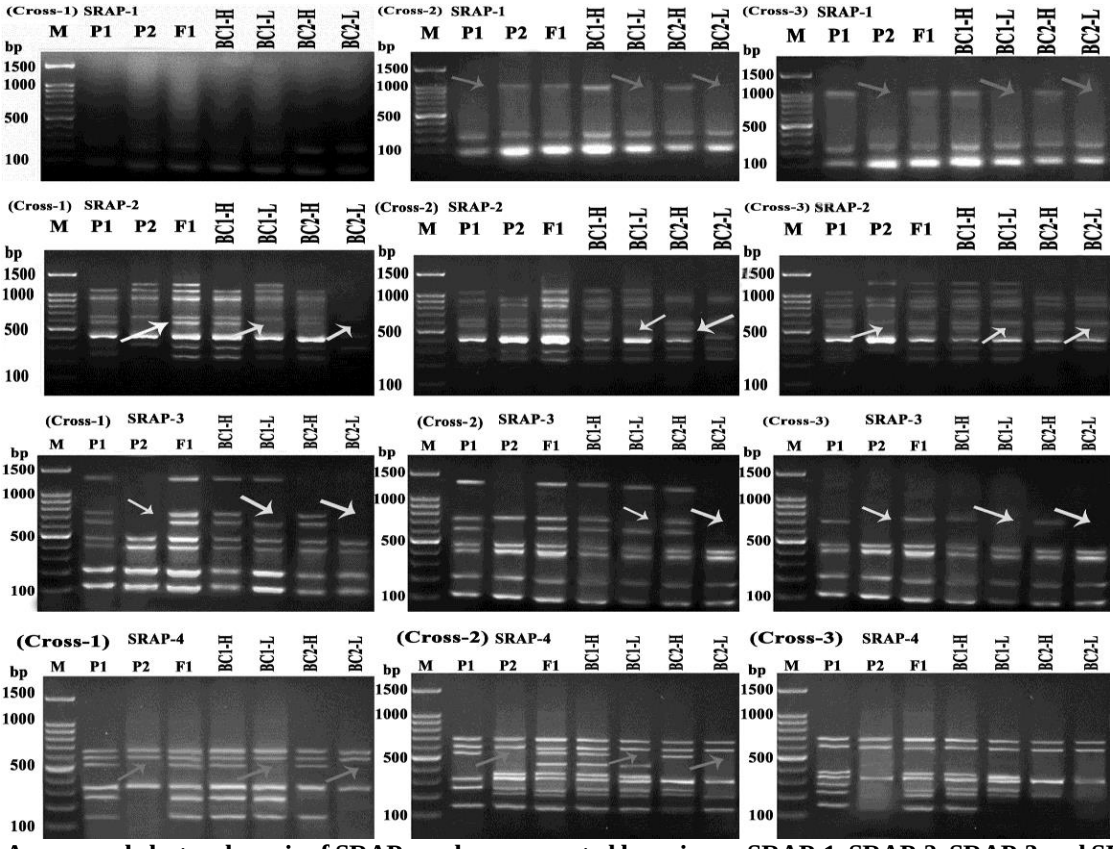

Fig. 1. Agarose gel electrophoresis of SRAP markers generated by primers SRAP-1, SRAP-2, SRAP-3 and SRAP-4 in the three parents Shakha-8, Sham-8, Line-6, their F1, high and low salt tolerance from BC_1 and BC_2.

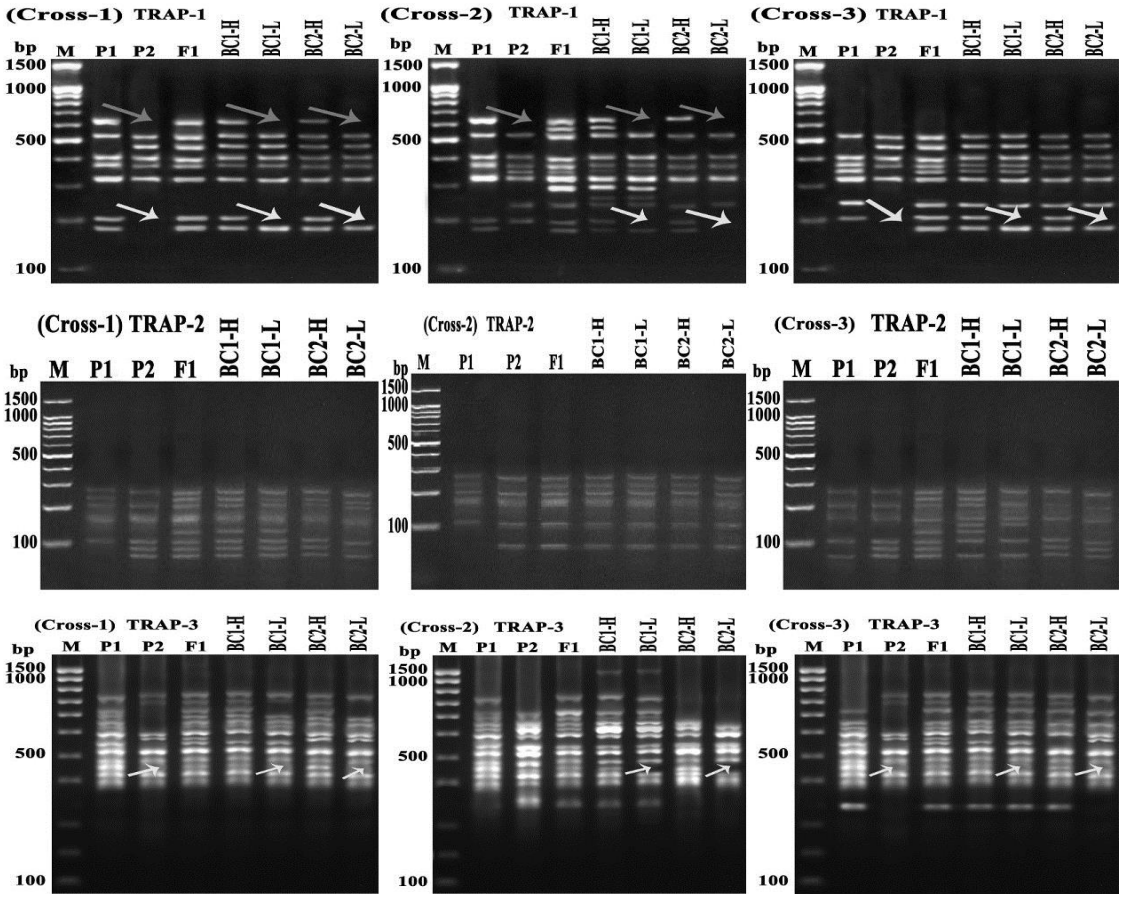

Fig. 2. Agarose gel electrophoresis of TRAP markers generated by primers TRAP-1, TRAP-2 and TRAP-3 in the three parents Shakha-8, Sham-8, Line-6, their F1, high and low salt tolerance from BC_1 and BC_2.

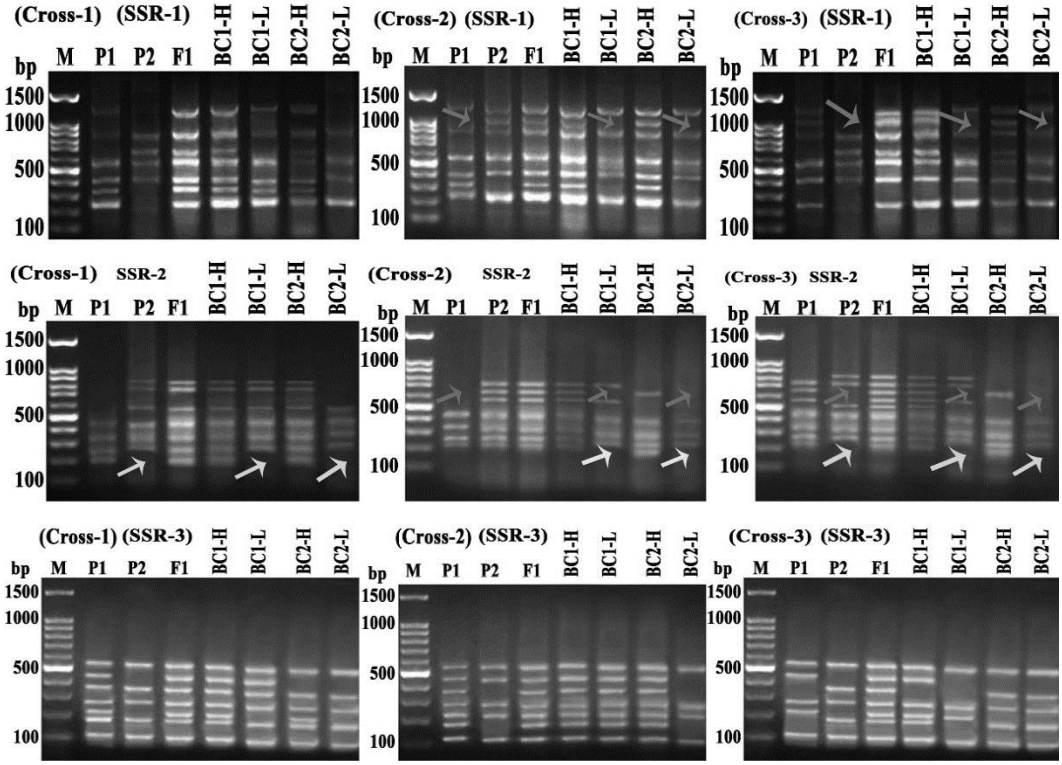

Fig. 3. Agarose gel electrophoresis of SSR markers generated by primers SSR-1, SSR-2 and SSR-3 in the three parents Shakha-8, Sham-8, Line-6, their F1, high and low salt tolerance from BC_1 and BC_2.

Identification of molecular markers associated with salt tolerance

Bulk segregant analysis

Ten primers of the three markers (4 SRAP, 3 TRAP and 3 SSR) that produced clear discrimination patterns between the three parental genotypes were used to distinguish between bulked DNA from high and low salinity tolerant within each BC populations.

The SRAP-2 primer combination, one strong polymorphic DNA fragment at 570 bp was presented only in F_1, tolerant BC_1 bulk, tolerant BC_2 bulk and two parents, Sakha-8 and Line-6 (resistant parents) for three crosses whereas missing in sensitive BC_1 and BC_2 bulks and Sham-8 (sensitive parent) for cross-1 (Sakha-8 x Sham-8) and cross-3 (Line-6 x Sham-8), as shown in Fig 1. In addition primer combination SRAP-3, produced a strong polymorphic band at 760 bp that was present only in the F_1, tolerant BC_1 bulk, tolerant BC_2 bulks and two parents, Sakha-8 and Line-6 (resistant parents) for three crosses, but not in the sensitive BC_1, BC_2 bulked DNA and Sham-8 (sensitive parent) for cross-1 (Sakha-8 x Sham-8) and cross-3 (Line-6 x Sham-8), as shown in Fig 1.

DNA band at molecular weight 205 bp which amplified by TRAP-1 primer combination was presented only in tolerant genotypes (Sakha-8, Line-6, F_1, and DNA bulk for BC_1 and BC2) whereas, this band not found in sensitive genotypes (BC_1, BC_2 bulk and Sham-8) (Fig. 2). In addition, TRAP-3 primer combination was generated a strong marker (band at 450 bp) only in the tolerant parent (Sakha-8, Line-6), F_1 plants and tolerant BC_1 and BC_2 bulks for three crosses (Fig. 2).

Of three SSR primer pairs, the SSR-2 primer, generated one DNA fragment at 215 bp, which was present only in F_1, tolerant DNA bulked BC_1 and BC_2 and tolerant parent (Sakha-8, Line-6) and were missing in sensitive parent (Sham-8) and sensitive BC_1 and BC_2 in three crosses (Fig. 3)

The five polymorphic bands SRAP-$2_{570bp,}$ SRAP-$3_{760bp,}$ TRAP-$1_{205bp,}$ TRAP-3_{450bp} and SSR-2_{215bp} which appeared only in tolerant genotypes may be considered as specific markers for salt tolerance. Such bands were which transmitted into BC generations from the two parents (Sakha-8 and Line-6) contributed to the improvement and upgrading of salt-tolerant trait. Thus, the results suggested that selection of plants contained alleles raising the value of this trait can be used in the breeding programs to enhance salt-tolerant trait, while the exclusion of plants that possess alleles decreasing salt-tolerant from the breeding program saves the effort and time needed to improve such trait.

The three DNA bands SRAP-1_{1055bp}, SSR-1_{1130bp} and SSR-2_{650bp} were observed only in F_1, tolerant DNA bulked BC_1 and BC_2 and tolerant parent (Line-6) in cross-2 (Sahka-8 x Line-6) and cross-3 (Line-6 x Sham-8) whereas not observed in all genotypes in cross-1 (Sahka-8 x Sham-8) and all sensitive genotypes in cross-2 (Sahka-8 x Line-6) and cross-3 (Line-6 x Sham-8) (Fig. 1 and 3). These finding confirmed that these tolerant alleles transmitted into tolerant BC populations from the tolerant parent Line-6 only.

The three DNA bands SRAP-4_{535bp}, TRAP-1_{655bp} were observed only in F_1, tolerant DNA bulked BC_1 and BC_2 and tolerant parent (Sakha-8) in cross-1 (Sahka-8 x Sham-8) and cross-2 (Sahka-8 x Line-6) whereas not observed in all genotypes in cross-3 (Line-6x Sham-8) and all sensitive genotypes in cross-1 (Sahka-8 x Sham-8) and cross-2 (Sahka-8 x Line-6) (Fig. 1 and 2). These finding confirmed that these tolerant alleles transmitted into tolerant BC populations from the tolerant parent Sakha-8 only.

Thus, the results suggested that the two parents, Sakha-8 and Line-6 contained salt-tolerant alleles could be used in the wheat breeding programs to improve this trait.

Similar conclusion was also reached by Moghaieb *et al.* (2011), Ahmad *et al.* (2013), El-Rawy and Youssef

(2014), Ghaedrahmati *et al.* (2014). El-Hendawy *et al.* (2019) and ELshafei *et al.* (2019).

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

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تمت دراسة تحليل متوسطات الأجيال لتحمل الملحية في القمح على عشائر الآباء والجيل الأول والثاني ، الهجين الرجعى الاول والثاني لثلاثة من الهجن. كان الهجين الأول بين الصنف سخا٨ المتحمل للملحية والصنف شام٨ الحساس، والهجين الثاني بين الأصناف المتحملة سخا٨ وسلالة٦، أما الهجين الثالث كان بين سلالة٦ وشام٨. تم تقييم الطرز تحت ظروف الكنترول وإجهاد الملحية (١٥٠ ملليمول كلوريد صوديوم) في مرحلتي الإنبات والبادرات باستخدام سبع صفات. لم ينطبق نموذج الإضافة- سيادة لجميع الصفات تحت معاملة الكنترول والملحية فى الهجن الثلاثة. يشير وجود التفوق وزيادة التباين الوراثي غير الإضافي لجميع الصفات عدا طول الساق، إلى أن طرق الانتخاب التقليدية لا تكون ذات جدوى لتحسين هذه الصفات. لذلك فإن تأخير الانتخاب إلى الأجيال المتقدمة أو إجراء التهجين بين المنتخبات الانعزالية متبوعا بالتلقيح الذاتي لجيل أو اثنين فإنه يمكن أن يعمل على تراكم الأليلات المرغوبة لتحسين هذه الصفات. وقد أوضح تحليل الواسمات الجزيئية أن أربعة فقط من بادئات الـ SRAP ، وثلاثة بادئات من الـ TRAP ، وثلاثة بادئات من الـ SSR أظهروا اختلافات في تعدد أشكال حزم الـ DNA الناتجة من التراكيب الوراثية المختبرة. ظهرت الحزم الخمس ($SRAP\text{-}2_{570bp}$, $SRAP\text{-}3_{760bp}$, $TRAP\text{-}1_{205bp}$, $TRAP\text{-}3_{450bp}$ and $SSR\text{-}2_{215bp}$) في الأصناف المتحملة للملحية فقط.. الواسمات الجزيئية التي تم تحديدها في هذه الدراسة تسمح باستخدام الواسمات الانتخابية في فحص وغربلة العشائر الانعزالية لصفة تحمل الملحية.