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Genetic Basis for Salt Stress Tolerance in Wheat through Using ISSR Markers

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ABSTRACT

This study was launched as a serious attempt to genetically improve in wheat crop with the aim of increasing its tolerance to salt stress under Egyptian conditions. The degrees of negative risks of salt stress began to increase over the past few years, which led to a reduction in agricultural areas for growing strategic crops, especially wheat. On this basis, it was necessary to make a scientific leap in the field of plant breeding and biotechnology with the aim of obtaining new wheat lines that are tolerant to dangerous environmental factor, besides, its high yields. Some promising wheat accessions included (Parents & Hybrids) were tested under normal and salinity conditions through evaluating agro-morphological, biochemical, salinity tolerance indices and physiological attributes related to salinity tolerance in this regard. Further, eleven ISSR primers were used to generate all genetically molecular differences among all wheat entries which confirmed highly tolerance and yielding. The final results obtained highly yield and salinity tolerance in five wheat hybrids besides, their parents depending on all data calculated in both experiments. Molecular markers showed polymorphism % closed to 100.0% among all wheat accessions using SR-08 and SR-45 primers besides, 34 unique bands.

Keywords: Wheat, salt Stress, ISSR primers, plant breeding.

1. Introduction

Wheat is the most important food crop locally and globally, as it represents the focus of food security for any country (El-Mouhamady *et al.,* 2019 & EL Sabagh *et al.,* 2021). The situation in Egypt is of great strategic importance represented in the strenuous attempts to advance this crop in light of the salinity of the soil and irrigation water crisis, which began to increase recently due to the limited irrigation water, which began to take a dangerous course, especially after the construction of the Grand Ethiopian Renaissance Dam. Moreover, the problem of climate change, especially the phenomenon of global warming, led to an increase in the frequency of water stress and consequently, this led to an increase in the rate of salt accumulation in the soil and irrigation water. The result of these variables had a negative impact on the productivity of important strategic crops such as wheat, rice and maize, as these crops represent an important source of calories and protein (Kizilgeci *et al.,* 2021 & Khatab *et al.,* 2021). Also, salt stress has very dangerous negative effects on all physiological, biological, biochemical and metabolic processes related to the germination and growth of various field crops and their productivity as well (Parida 2005; Nessem & Kasim 2019). Rather, it negatively affects the rate of National Agricultural Production for countries that base their national product on agricultural production (El-Mouhamady *et al.,* 2012b & Khatab *et al.,* 2021b). The estimates of genetic variation and some physiological and yield components attributes associated with salt stress tolerance were estimated by (Al-Khaishany *et al.,* 2018). The low rate of water needed to wash away excess salts in the soil destroys the metabolism process and affects the ionic, hormonal balance and the anti-ions necessary for the survival of cells (Neumann 1977). As, the wheat crop is grown in vast areas globally

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and it may acquire more areas than any other crop, due to its strategic importance in general. It is mainly grow in Canada, France, China, Russia, Ukraine besides, the United States of America, as they are mainly exporting countries for this strategic crop (Shewry 2009). The percentage of lands affected by salt stress globally reaches 20% and this percentage may increase with time due to the phenomenon of climate change and global warming, which raises the temperature of the atmosphere and causes water deficit conditions, as well as unfair human activities (Arora 2019). Therefore, the losses caused by the highly level of salinity in the soil and irrigation water may reach 50% of the final output of any crop (Acquaah 2007). Also, the dangerous effects of water and salt stresses have been the focus of a large number of researchers such as (El-Mouhamady, 2003 & 2009) in rice, (khatab *et al.,* 2017) in sorghum (Khatab *et al.,* 2019) in barley and (El-Mouhamady & Ibrahim 2020) in wheat. The following is a quick review of the most important results of research and studies that dealt with the improvement and development of wheat genotypes to salt stress tolerance whether by traditional plant breeding methods or through genetic engineering and biotechnology. The final total productivity of crops in general and wheat in particular is low and severely affected under salt stress conditions. This is due to the lack of information on the physiological, biochemical and genetic mechanisms responsible for tolerance to salt stress (Kumar *et al.,* 2017). SOS1, HKT and NHX genes succeeded in producing a high degree of response to salt stress tolerance in the Turkey wheat genotype and this represents an important reflection of the antioxidant activity in this variety through increasing the level of CAT and SOD enzymes more than any other variety, which gave it a high degree of tolerance to salt stress (Tounsi *et al.,* 2017). The statistical model used to study the tolerance of wheat to salt stress that fully corresponds with each of all morphological, physiological, and biochemical attributes will be more successful and comfortable than listing the results for sensitive, medium sensitive and more tolerant strains of salt stress in this context (Al-Ashkar *et al.,* 2019). Molecular markers using 17 SS primer was used to examine and determine the different genotypes of wheat and the degree of their response to salt stress tolerance. Thus, these markers proved to be highly effective in detecting future tolerance in any wheat accession through marker assisted-selection technique (Al-Ashkar *et al.,* 2020). One of the most famous tangible and observed tolerance indices of wheat genotypes under salt stress conditions is accumulating of some organic acids which have a close relationship with salt stress tolerance, such as proline content and this is conclusive evidence that cannot be doubted about the importance of proline acid in raising the degree of tolerance of plants to abiotic stress especially salt stress (El-Saber 2021). The standardization of phenotypic and molecular markers through using SSR primers had the greatest advantage in providing a genetic database that provides wheat breeders with the opportunity to select the most tolerant genotypes for salt stress (Urbanaviciute *et al.,* 2023). After all that has been presented, it is possible to briefly clarify the aim of this investigation, which is centered on trying to devise new wheat accessions that are highly productive and tolerant to salt stress through traditional and novel plant breeding methods. Thus, it will be a fruitful trend to increase the productivity of the Egyptian wheat crop to bridge the large gap in the production of Egyptian bread.

2. Materials & Methods

2.1. Materials

The present investigation was carried out in two locations (The normal and salinity conditions) in 2020 season. Each location was included using 15 wheat entries (the five wheat parents and their ten F1 wheat crosses) through half diallel analysis. Further, the five wheat cultivars with various reaction for salinity tolerance. The first location was normal or control experiment and conducted at Al-Noubaria Farm in Beheira Governorate. While, the saline treatment was carried out in the El-Sirw farm in Damietta Governorate, as it is a naturally saline soil. The five wheat parents were Sakha 8, Misr 1, Sakha 94, Gimeaza 11 and Gimeaz 12 where the first two cultivars were tolerance for salinity stress and the other wheat cultivars were moderately, table (1).

Table 1: Classification of the five Wheat Parents used in a half diallel analysis.

Serial No.	Names of Genotypes	Origin	Salinity tolerance
1 or (P1)	Sakha 8	Egypt	Tolerance
2 or (P2)	Misr 1	Egypt	Tolerance
3 or (P3)	Sakha 94	Egypt	Moderate
4 or (P4)	Gimeaza 11	Egypt	Moderate
5 or (P5)	Gimeaza 12	Egypt	Moderate

2.1.1. Sowing

The five wheat parents were sown in three planting dates with 7 days interval in order to overcome the differences in flowering time among parents for crossing in seasons 2018 and 2019 to obtain a large quantity of first generation hybrid seeds. All entries (parents and their F1 crosses) were grown under normal and salinity conditions in a randomized complete block design with three replicates for each experiment in season 2020. The chemical analysis of both soil locations was found in table (2).

Table 2: - Chemical analysis of both Soil locations (normal and saline soils) using in this study.

Characteristics	Normal Soil (Al-Noubaria Farm)	Saline water (El-Sirw farm)
EC (dS/m)	2.71	1.38
pH (1:2.5)	8.22	8.51
Ca++	1.93	15.62
Mg++	1.33	12.62
Na+	7.92	65.12
K+	0.48	0.27
$CO3^{--}$	0.05	0.15
$HCO3^{-}$	1.82	4.15
Cl^{-}	13.77	58.11
$SO4^{-}$	1.17	51.32

2.1.2. Studied traits

Fifty plants were taken from each genotype for the two experiments (normal and saline treatments) to evaluate the following traits.

1) Number of filled grains per panicle

Filled grains of the main panicle with separated and counted, 2) 1000-grain weight (g):- It was recorded as the weight of 1000 random filled grains per plant, 3) Grain yield per plant (g):- was recorded as the weight of grain yield of each individual plant, and adjusted to 14% moisture content, 4) The proline content: - was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline / ml C m1 toluence) / 115.5 µg / µ mole] / [(g sample/5)] = µ moles proline / g of fresh weight material. The results related with proline content are average values at least 3-4 samples for each species, according to Chinard (1942) and modified method by Bates *et al.,* (1973).

2) Glycine betaine content: - It was carried out according to the method of Grieve & Grattan (1983).

3) Osmotic adjustment: - It was determined as follows: -

$$\frac{\text{O.P. x R.W.C.}}{100}(\text{Normal}) - \frac{\text{O.P x R.W.C.}}{100}(\text{drought})^{100};$$

Where: O.P= Osmotic pressure, R.W.C. = Relative water content.

2.2. Methods

2.2.1. Statistical analysis

All calculated data from all studied traits under the two experiments were analyzed using half diallel analysis by (Griffing 1956) model I, method II including heterosis over better-parent, general and

specific combining ability effects where **GCA/SCA ratio: -** MSe of GCA-MS error term /Number of parent + 2/ MSe of SCA-MS error term, respectively.

2.2.2. Estimation of tolerance indices

All tolerance indices were estimated according to (Fischer & Maurer, 1978; Bouslama & Schapaugh, 1984; Fernandez 1992; Gavuzzi *et al.,* 1997 & Golestani & Assad 1998) as follows in table (10):- 1):- GYP: Is meaning the grain yield/plant for the control experiment, 2):- GYS: Is meaning the grain yield/plant for the salinity experiment, 3):- YSI: Is meaning yield stability index = YS/YP where: YS the average of yield under stress and YP= The average of yield under the control experiment, 4):- YI: Is meaning yield index (YS for each genotype/mean of YS for all genotypes), 5):- MP: Is means (Average yield for both trials): YS + YP/2 , 6):- STI: Is meaning salinity tolerance index (YP X YS/ (mean of YP) 2 , 7):- GMP: (YP X YS) 0.5 , 8):- YR: Is meaning yield reduction (1-YS/YP). 9):- SSI: Is meaning salinity susceptibility index = (1-YS/YN)/D where YS = mean yield under salt stress, YN = mean yield under control or normal condition, and D = environmental stress intensity= 1-(mean yield of all genotypes under stress/mean yield of all genotypes under irrigated conditions). Note: - Osmotic adjustment was conducted according to (Jones & Turner 1978).

2.3. Molecular Characterization

2.3.1. Genomic DNA extraction and PCR condition

Total genomic DNA of all samples was extracted from twelve green wheat leaves (The five parents and the best seven F1 crosses) using Qiagen DNeasy Plant Minikit following the protocol of the manufacturer (Qiagen Inc, Valencia, CA). The quality of the extracted DNA was assessed on agarose gel electrophoresis. PCR was performed using ten preselected ISSR primers based on their ability to generate reproducible and informative amplification patterns. Amplification reactions were carried out in Biometra T One Thermal Cycler (Analytik Jena, Jena, Germany). PCR amplification was performed in 25 µl reaction mix which contained 20-30 ng DNA template, 10 pmole of each primer, 2.5 µl of 2mM Thermo dNTPs, 5 µl of 5x Promega Green GoTaq Flexi Reaction Buffer, 2.5 µl of 25 mM Promega $MgCL_2$ and 0.125 µl of 5 U/µl Promega GoTaq Flexi DNA polymerase. The reaction was assembled on ice, amplification was performed at certain conditions as follows: an initial denaturing step at 94 ^{0}C for 5 min followed by 35 cycles at 94 ^{0}C for 30 sec, annealing at 50 ^{0}C for 1 min, an extension at 72 ^{0}C for 1 min and final extension at 72 ^{0}C for 7 mins. The PCR products was assessed on 1.6% agarose gel (Sambrook *et al.,* 1989; Zietkiewicz *et al.,* 1994 & Gezahegn *et al.,* 2010). Banding profile of ISSR were scored using Labimage program and the polymorphism percentage was estimated as follow :- Percent of polymorphism = (Number of polymorphic bands/Total Number of Bands) X 100.

The best seven crosses were: - H1: Sakha 8 X Sakha 94, H2: Sakha 8 X Gimeaza 11, H3: Sakha 8 X Gimeaza 12, H4: Misr 1 X Sakha 94, H5: Misr 1 X Gimeaza 12, H6: Sakha 94 X Gimeaza 12 and H7: Gimeaza 11 X Gimeaza 12 respectively.

Note:- Molecular Sizes of marker used in analyses were as follow: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 3000 bp. Also: - T.B: Total bands, M.B: Monomorphic bands, P.B: Polymorphic bands, U.B or P.S.M: Unique bands or positive specific marker, P%: Polymorphism percentage and R.S (bp): Range size.

Table 3: Name and sequences of the eleven selected ISSR primers used in PCR profile analysis.

Primer Code	Sequence(5'→3')	Abbrev.	Mer
SR-01	ACACACACACACACC	(AC)8C	17
SR-03	ACACACACACACACT	(AC)8T	17
SR-04	ACACACACACACACYA	(AC)8YA	18
SR-08	AGAGAGAGAGAGGG	(AG)6GG	14
SR-11	AGAGAGAGAGAGAGAGT	(AG)8T	17
SR-12	AGAGAGAGAGAGAGAGYA	(AG)8YA	18
SR-13	AGAGAGAGAGAGAGAGYC	(AG)8YC	18
SR-21	CACACACACACACACAT	(CA)8T	17
SR-40	GTGTGTGTGTGTGTGTYG	(GT)8YG	18
SR-41	TCTCTCTCTCTCTCTCA	(TC)8A	17
SR-45	CGCGATAGATAGATAGATA	CGC(GATA)4	19

2.3.2. Data Handling and cluster analysis (Phylogenetic Tree)

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise components of the ten wheat accessions based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients according to Jaccard (1908). The similarity coefficients were then used to construct dendrograms, using the un weighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

3. Results

3.1. Interaction & Mean Performance

Quantitative genetics and plant breeding have played an effective and major role in creating a constructive boom in the advancement of field crops to confront environmental and biotic stresses, especially in the last five decades in Egypt. It contributed significantly to raising the degree of tolerance in wheat crop to water and salt stress to a significant degree, especially in areas close to sea water and coastal areas affected by the high level of salt deposition, especially with the decline in the amount of irrigation water, which placed Egypt in the water poverty belt. Further, this trend in breeding parallels and integrates with modern science methods represented by new genetic techniques in discovering genes for tolerance and resistance to environmental and biotic stresses, such as biotechnology and molecular markers, which helped in transferring these genetic mechanisms from tolerant wild lines to sensitive Egyptian varieties during breeding programs in a very record time compared to the length of time breeding in the past. Data observed in table (4) detected that highly significant variation were showed among all wheat entries under study for both conditions. These results indicated that the biggest role of this test to indicate the tolerance degrees in all wheat accessions under normal and salinity conditions. Also, the first three wheat parents were achieved highly rank of salinity tolerance for all traits under study in both conditions besides, the wheat accessions; (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 and P4 X P5) for the same treatments, table (5). Where, these promising entries were succeed for showed the biggest level of salinity tolerance depending on all data of yield components besides, physiological and biochemical attributes under salinity treatment compared to the standard experiment.

3.2. Heterosis over better Parent

Results obtained in table (6) detected that the most desirable wheat hybrids recorded significant and highly significant positively values and highly level of salinity tolerance for all yield components, physiological and biochemical attributes studied under both conditions were the crosses; (P1 X P3, P1 X P4, P1 X P5, P2 X P3 and P2 X P5), respectively. On the other hand, the rest hybrids were achieved negatively and highly significant values for the same traits under the same conditions.

3.3. Combining Ability effects

The first three wheat parents were recorded significant and highly significant positively values of GCA effects of all attributes under testing for the normal and saline treatments. While, the rest of the other results for this parameter were in the opposite negative direction for the fourth and fifth parents under the same measurements and conditions, table (7). Also, the same promising hybrids (P1 X P3, P1 X P4, P1 X P5, P2 X P3 and P2 X P5) were exhibited highly significant positively data of SCA effects for all yield, physiological and biochemical attributes for the two treatments. Further, the rest crosses achieved negative and highly significant results for the same attributes and measurements described previously, table (7).

3.4. Salinity Tolerance Indices

Results of all previous genetic measurements have proven the extent of genetic improvement and progress achieved in raising the degree of salt stress tolerance in some of the superior and promising wheat accessions. Moreover, these promising materials were the same tolerant genotypes that were previously mentioned in genetic measurements. Further, it succeeded beyond any doubt in reducing the final losing in the final output under salting treatment compared to the standard experiment. Also, it has

Table 4: Mean Squares of the half diallel analysis for all studied traits under the control and salinity conditions.

S.O.V	D.F	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline Cont.		Glycine betaine Cont.		Osmotic adjustment
		N	S	N	S	N	S	N	S	N	S	
Reps	2	1.38	2.71	1.56	1.93	1.32	1.15	2.61	1.55	1.42	1.38	0.78
Genotypes	14	11.45**	32.67**	6.56**	7.34**	19.45**	22.12**	17.45**	31.45**	24.34**	26.05**	17.67**
GCA	4	312.45**	126.44**	78.45.54**	55.13**	156.21**	188.09**	256.12**	308.41**	119.05**	128.16**	498.12**
SCA	10	117.03**	89.04**	33.04**	28.32**	103.34**	67.55**	133.22**	185.71**	77.14**	93.04**	285.93**
Error	28	1.45	1.22	1.17	1.63	1.05	1.42	1.38	1.43	1.78	1.55	1.04
Error term		0.48	0.40	0.39	0.54	0.35	0.47	0.46	0.47	0.59	0.51	0.34
GCA/SCA		5199.5	125.99	371.71	218.36	2326.26	1.804.03	4851.23	8322.70	1301.75	2102.0	20.74

Table 5: Mean performances of all studied traits in all wheat accessions tested under the control and salinity conditions

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline Content)		Glycine Betaine Content		Osmotic adjustment
	N	S	N	S	N	S	N	S	N	S	
P1	**71.13**	**63.07**	**51.32**	**40.56**	**46.32**	**38.05**	**55.19**	**44.32**	**63.42**	**51.07**	**0.44**
P2	**66.56**	**57.91**	**55.41**	**42.23**	**48.15**	**36.08**	**58.93**	**39.42**	**60.18**	**47.38**	**0.48**
P3	**41.45**	**28.95**	**50.08**	**39.11**	**44.11**	**29.22**	**51.08**	**40.12**	**54.13**	**44.18**	**0.49**
P4	48.67	22.11	38.14	22.09	33.19	20.07	28.22	12.53	36.04	23.67	1.12
P5	44.15	31.02	32.08	19.54	36.24	19.88	32.05	19.44	33.14	20.05	1.38
P1 X P2	37.55	19.42	36.03	17.55	28.41	16.35	29.04	15.21	29.04	15.67	1.74
P1 X P3	**79.33**	**66.87**	**62.15**	**54.77**	**58.93**	**44.13**	**63.13**	**52.14**	**70.04**	**59.12**	**0.19**
P1 X P4	**82.05**	**69.04**	**68.18**	**52.22**	**62.08**	**41.05**	**70.03**	**59.22**	**83.24**	**71.45**	**0.22**
P1 X P5	**77.14**	**68.03**	**71.03**	**58.15**	**56.04**	**42.03**	**61.92**	**50.12**	**89.79**	**73.15**	**0.31**
P2 X P3	**84.11**	**72.05**	**65.02**	**55.04**	**61.05**	**52.07**	**73.24**	**58.14**	**80.14**	**66.35**	**0.17**
P2 X P4	31.02	18.14	28.38	16.32	28.95	17.31	38.22	17.94	24.18	11.07	2.11
P2 X P5	**80.03**	**71.13**	**73.28**	**60.09**	**55.11**	**47.08**	**81.07**	**63.41**	**103.34**	**92.13**	**0.27**
P3 X P4	30.19	16.55	34.08	21.15	38.02	22.05	25.48	13.21	30.04	16.55	1.84
P3 X P5	34.02	17.62	29.68	14.59	26.44	14.31	23.05	11.05	26.11	10.34	1.92
P4 X P5	40.23	17.25	31.17	20.03	35.18	21.03	22.03	10.84	31.08	18.73	2.03
LSD at 0.05	1.72	1.53	1.50	1.77	1.42	1.65	1.63	1.66	1.85	1.72	1.41
LSD at 0.01	2.49	2.22	2.17	2.57	2.06	2.40	2.36	2.40	2.68	2.50	2.05

Table 6: Heterosis over better-parent for the 10 wheat crosses obtained from half diallel analysis in all studied traits under both conditions.

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline content		Glycine betaine content		Osmotic adjustment
	N	S	N	S	N	S	N	S	N	S	
P1 X P2	-47.20**	-69.20**	-34.97**	-58.44**	-40.99**	-57.03**	-50.72**	-65.68**	-54.21**	-69.31**	295.45**
P1 X P3	**11.52****	**6.02****	**12.16****	**14.84****	**27.22****	**15.97****	**14.38****	**17.64****	**10.43****	**15.76****	**-0.56****
P1 X P4	**15.39****	**9.46****	**32.85****	**28.74****	**34.02****	**7.88****	**26.88****	**33.61****	**31.25****	**39.90****	**-50.0****
P1 X P5	**8.44****	**7.86****	**75.12****	**43.36****	**20.98****	**10.45****	**12.19****	**13.08****	**26.36****	**29.91****	**-29.54****
P2 X P3	**26.36****	**24.41****	**17.34****	**30.33****	**26.79****	**44.31****	**24.28****	**44.91****	**33.16****	**40.03****	**-64.58****
P2 X P4	-53.39**	-68.62**	-48.78**	-61.35**	-39.87**	-52.02**	-35.14**	-54.49**	-59.82**	-76.63**	339.58**
P2 X P5	**20.23****	**22.82****	**32.25****	**42.29****	**14.45****	**30.48****	**37.56****	**60.85****	**71.71****	**94.44****	**-43.75****
P3 X P4	-37.97**	-42.83**	-31.94**	-45.92**	-13.80**	-24.53**	-50.11**	-67.07**	-44.50**	-62.53**	275.51**
P3 X P5	-22.94**	-43.19**	-40.73**	-62.69**	-40.05**	-51.02**	-54.87**	-72.45**	-51.76**	-76.59**	291.83**
P4 X P5	-17.34**	-44.39**	-18.27**	-9.32**	-2.92**	4.78**	-31.26**	-44.23**	-13.76**	-20.87**	81.25**
LSD at 0.05	1.72	1.53	1.50	1.77	1.42	1.65	1.63	1.66	1.85	1.72	1.41
LSD at 0.01	2.49	2.22	2.17	2.57	2.06	2.40	2.36	2.40	2.68	2.50	2.05

Table 7: GCA and SCA effects in wheat accessions for the two conditions.

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline content		Glycine Betaine Content		Osmotic adjustment
	N	S	N	S	N	S	N	S	N	S	
P1	14.22**	12.98**	23.14**	19.25**	34.11**	29.05**	41.25**	33.18**	45.20**	13.96**	7.32**
P2	17.32**	10.05**	15.08**	17.44**	20.57**	14.32**	11.67**	16.42**	21.09**	24.15**	5.44**
P3	11.05**	17.44**	11.23**	9.55**	9.42**	10.05**	25.80**	51.90**	14.55	17.32**	13.52**
P4	-25.43**	-22.44**	-36.54**	-20.79**	-43.57**	-31.8**	-62.45**	-59.33**	-54.16**	-15.36**	-14.88**
P5	-17.16**	-18.03**	-12.91**	-25.48**	-20.53**	-21.44**	-16.27**	42.17**	-26.68**	-10.92**	-11.40**
LSD at 0.05 (gi)	**1.23**	**1.11**	**1.56**	**1.39**	**3.45**	**2.77**	**5.52**	**4.77**	**2.54**	**1.67**	**1.32**
LSD at 0.01 (gi)	**1.57**	**1/34**	**1.94**	**2.04**	**4.08**	**3.47**	**7.13**	**6.88**	**3.14**	**2.19**	**1.79**
P1 X P2	-10.11**	-7.73**	-55.12**	-25.94**	-17.88**	-25.40**	-61.23**	-90.08**	-170.14**	-83.15**	-17.45**
P1 X P3	8.32**	6.55**	14.62**	28.05**	41.15**	28.45**	77.23**	68.04**	78.12**	67.04**	10.03**
P1 X P4	23.01**	13.06**	10.07**	19.11**	28.30**	62.05**	52.09**	37.66**	103.11**	97.04**	23.83**
P1 X P5	9.77**	10.04**	32.47**	24.03**	19.34**	14.85**	39.21**	42.13**	63.19**	59.12**	12.87**
P2 X P3	5.45**	7.03**	104.27**	86.63**	12.47**	23.11**	51.12**	28.27**	50.06**	39.28**	18.05**
P2 X P4	-14.25**	-6.28**	-113.88**	-34.15**	-13.05**	-17.41**	-55.14*	-32.17**	-55.80**	-119.46**	-21.15**
P2 X P5	14.08**	21.05**	41.05**	29.03**	10.08**	14.35**	11.67**	13.98**	100.05**	108.32**	39.43**
P3 X P4	-6.27**	-14.05	-7.05**	-26.76**	-31.48**	-13.14**	-89.14**	-14.28**	-49.23**	-68.64**	-38.12**
P3 X P5	-22.04**	-9.67**	-16.11**	-71.15**	-31.15**	-54.82**	-12.82**	-30.08**	-78.25**	-50.14**	-16.78**
P4 X P5	-7.96**	-20.0**	-10.32**	-28.85**	-17.78**	-32.04**	-12.99**	-23.47**	-41.11**	-49.41**	-10.71**
LSD at 0.05 (Sij)	**1.05**	**1.17**	**3.45**	**2.98**	**6.32**	**4.59**	**8.71**	**6.32**	**4.32**	**1.94**	**3.57**
LSD at 0.01 (Sij)	**1.22**	**1.32**	**4.62**	**3.76**	**7.29**	**5.13**	**9.55**	**7.19**	**5.78**	**2.78**	**4.55**

already been proven to be less sensitive to salt stress, unlike the other rest whet entries under the same conditions and attributes under study, table (8).

Table 8: Salinity tolerance indices in wheat entries for the two experiments.

Genotypes	1	2	3	4	5	6	7	8	9
P1	46.32	38.05	0.82	1.23	42.18	0.91	41.52	**0.18**	**0.60**
P2	48.15	36.08	0.74	1.17	42.11	0.90	41.68	**0.26**	**0.86**
P3	44.11	29.22	0.66	0.95	36.66	0.66	35.90	**0.34**	1.13
P4	33.19	20.07	0.60	0.65	26.63	0.34	25.80	0.40	1.33
P5	36.24	19.88	0.54	0.64	28.06	0.37	26.84	0.46	1.53
P1 X P2	28.41	16.35	0.57	0.53	22.38	0.24	21.55	0.43	1.43
P1 X P3	58.93	44.13	0.74	1.43	51.53	1.35	50.99	**0.26**	**0.86**
P1 X P4	62.08	41.05	0.66	1.33	51.56	1.32	50.48	**0.34**	1.13
P1 X P5	56.04	42.03	0.75	1.36	49.03	1.22	48.53	**0.25**	**0.83**
P2 X P3	61.05	52.07	0.85	1.69	56.56	1.65	56.38	**0.15**	**0.50**
P2 X P4	28.95	17.31	0.59	0.56	23.13	0.26	22.38	0.41	1.36
P2 X P5	55.11	47.08	0.85	1.53	51.09	1.34	50.93	**0.15**	**0.50**
P3 X P4	38.02	22.05	0.57	0.57	30.03	0.43	28.95	0.63	1.43
P3 X P5	26.44	14.31	0.54	0.46	20.37	0.19	19.45	0.46	1.53
P4 X P5	35.18	21.03	0.59	0.68	28.10	0.38	27.19	0.61	1.36

3.5. Molecular Characterization

11 ISSR markers in this study were used in determining among 12 wheat genotypes. These eleven primers generated 117 amplicons, (Table 9; Fig.1). The highest levels of polymorphism % were observed in the primers; SR-08 (100.0%) and SR-45 (100.0%), followed by the primer SR-12 (87.5%), followed by the primer SR-40 (85.71%) and then followed by the primer SR-04 (83.33%), respectively. Data shown in table (10) detected that the highest number of unique bands were observed in primer SR-03 (10), followed by the primers; SR-01, 8 and 12 where the number were five markers for each one of them, followed by the primers; SR-04 and 11 (4), followed by the primer; SR-40 (2) and then followed by the primer SR-13 (1), respectively. For example not limited, the biggest number of unique markers was found in the primer; SR-03 as follows; six positive specific markers in in the hybrid number three at molecular weights of 897 bp, 641 bp, 542 bp, 496 bp, 456 bp and 433 bp, respectively. While, four negative markers were showed for the same primer for hybrid 3 at molecular weights of 971 bp, 836 bp, 584 bp and 467 bp. On the other hand, the primer SR-13 was generated the lowest positive specific markers (1) for the third cross at molecular weight of 153 bp, table (10).

Table 9: Band variation and polymorphism percentage in the twelve wheat genotypes using 11 ISSR markers

No.	ISSR primers	T.B	M.B	P.B	U.B	P %	R.S (bp)	Sequence
1	**SR-01**	13	4	9	**5**	**69.23%**	1002-250	ACACACACACACACACC
2	**SR-03**	19	8	**11**	10	**57.89%**	1409-245	ACACACACACACACACT
3	**SR-04**	6	1	**5**	3	**83.33%**	838-332	ACACACACACACACACYA
4	**SR-08**	15	0	15	5	**100.0%**	1463-292	AGAGAGAGAGAGGG
5	**SR-11**	13	3	10	3	**76.92%**	1358-289	AGAGAGAGAGAGAGAGT
6	**SR-12**	8	1	7	**5**	**87.50%**	1193-269	AGAGAGAGAGAGAGAGYA
7	**SR-13**	6	3	3	1	**50.0%**	556-153	AGAGAGAGAGAGAGAGYC
8	**SR-21**	8	8	0	0	**0.0%**	863-267	CACACACACACACACAT
9	**SR-40**	7	1	6	**2**	85.71%	834-266	GTGTGTGTGTGTGTGTYG
10	**SR-41**	3	2	1	0	33.33%	1085-622	TCTCTCTCTCTCTCTCA
11	**SR-45**	19	0	19	0	100.0%	1543-267	CGCGATAGATAGATAGATA
Total		117	31	86	34	73.50%	1543-153	

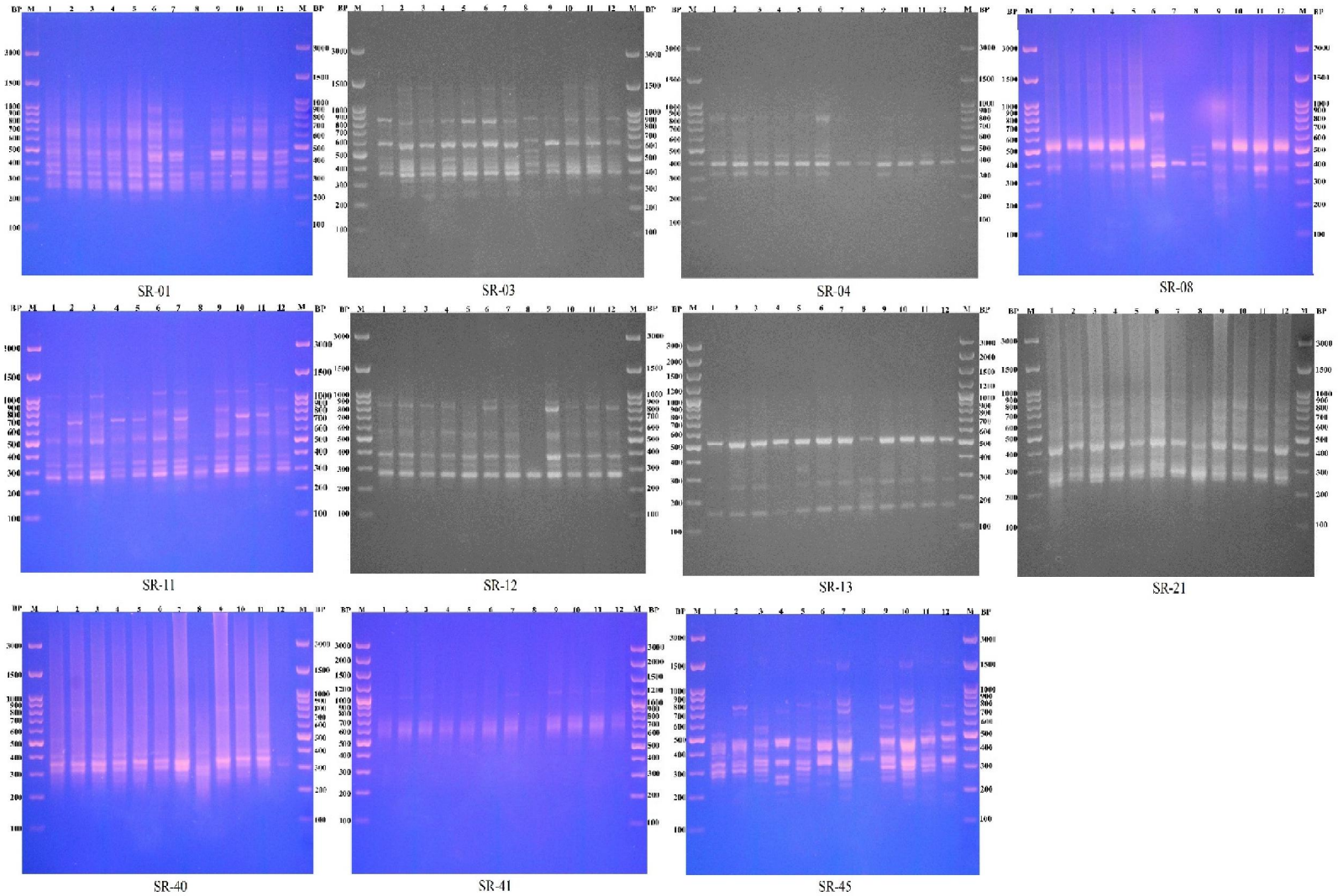

Fig. 1: ISSR primers used in the twelve wheat genotypes namely;-SR1, 3, 4, 8, 11, 12, 13, 21, 40, 41 and 45

Table 10: Mapping of unique amplicons in wheat genotypes.

ISSR Primers	MS(bp)	Parent 1	Parent 2	Parent 3	Parent 4	Parent 5	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	Hybrid (6)	Hybrid (7)	(P or N) Marker
SR-01	767	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	675	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	455	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	306	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	272	-	+	+	+	+	+	+	+	+	+	+	+	Negative (P1)
SR-03	971	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	897	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	836	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	641	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	584	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	542	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	496	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	467	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	456	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	433	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
SR-04	463	+	+	+	+	+	+	-	+	+	+	+	+	Negative (H2)
	402	+	+	+	+	+	+	-	+	+	+	+	+	Negative (H2)
	332	+	+	+	+	+	+	-	+	+	+	+	+	Negative (H2)
SR-08	1463	-	-	-	-	-	+	-	-	-	-	-	-	Positive (H1)
	1216	-	-	-	-	-	+	-	-	-	-	-	-	Positive (H1)
	1084	-	-	-	-	-	+	-	-	-	-	-	-	Positive (H1)
	860	-	-	-	-	-	+	-	-	-	-	-	-	Positive (H1)
	473	-	-	-	-	-	+	-	-	-	-	-	-	Positive (H1)
SR-11	1358	-	-	-	-	-	-	-	-	-	-	+	-	Positive (H6)
	879	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	593	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
SR-12	1193	-	-	-	-	-	-	-	-	+	-	-	-	Positive (H4)
	855	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	568	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	382	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	328	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
SR-13	153	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
SR-40	416	+	+	+	+	+	+	+	+	+	+	+	-	Negative (H7)
	364	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
Total														15 (Positive) + 19 (Negative)

3.6. Similarity Indices and Cluster Analysis

Results shown in table (11) recorded 55 relationships within 12 wheat entries detected about similarity. Data were 0.436 to 0.880 with an average of 0.658. The lowest rank of similarity was (0.436) among (P4 & H3) and the highest limit was (0.880) between (P1 & P2). Other high similarity values were obtained in this study for example not limited among (P1 & P3) (0.837), (P2 & P3) 0.822) and (H2 & H5) (0.818). These results indicated the essential role of correlated indices of all wheat accessions and its role of salt tolerance in the future through the steps of traditional and modern techniques of genetics.

Data of phylogenetic tree (Fig, 2) detected the result of 12 wheat genotypes were consisted two main cluster where the first one was (H3) only. While, the second cluster had two sub-cluster. The first one was (H1) only and the second sub-cluster divided into two sub-sub-cluster. Where, the first one was (H7) only. While, the second sub-sub-cluster were included the rest wheat relationships namely; (P1 & P2), (P1 & P3), (P4 & P5), (H4 & H6), (H5 & H6) and (H2) only.

Table 11: Similarity % of Wheat Entries within using ISSR markers.

Similarity %	P1	P2	P3	P4	P5	H1	H2	H3	H4	H5	H6	H7
P1	1.0											
P2	**0.880**	1.0										
P3	0.837	0.822	1.0									
P4	0.779	0.827	0.827	1.0								
P5	0.784	0.852	0.791	0.858	1.0							
H1	0.690	0.717	0.70	0.666	0.724	1.0						
H2	0.673	0.739	0.720	0.722	0.747	0.676	1.0					
H3	0.472	0.458	0.458	**0.436**	0.447	0.460	0.446	1.0				
H4	0.780	0.787	0.806	0.715	0.757	0.775	0.726	0.440	1.0			
H5	0.755	0.822	0.802	0.786	0.771	0.666	0.818	0.428	0.787	1.0		
H6	0.786	0.813	0.813	0.758	0.782	0.693	0.731	0.468	0.817	0.833	1.0	
H7	0.761	0.730	0.692	0.655	0.663	0.666	0.648	0.460	0.775	0.730	0.741	1.0

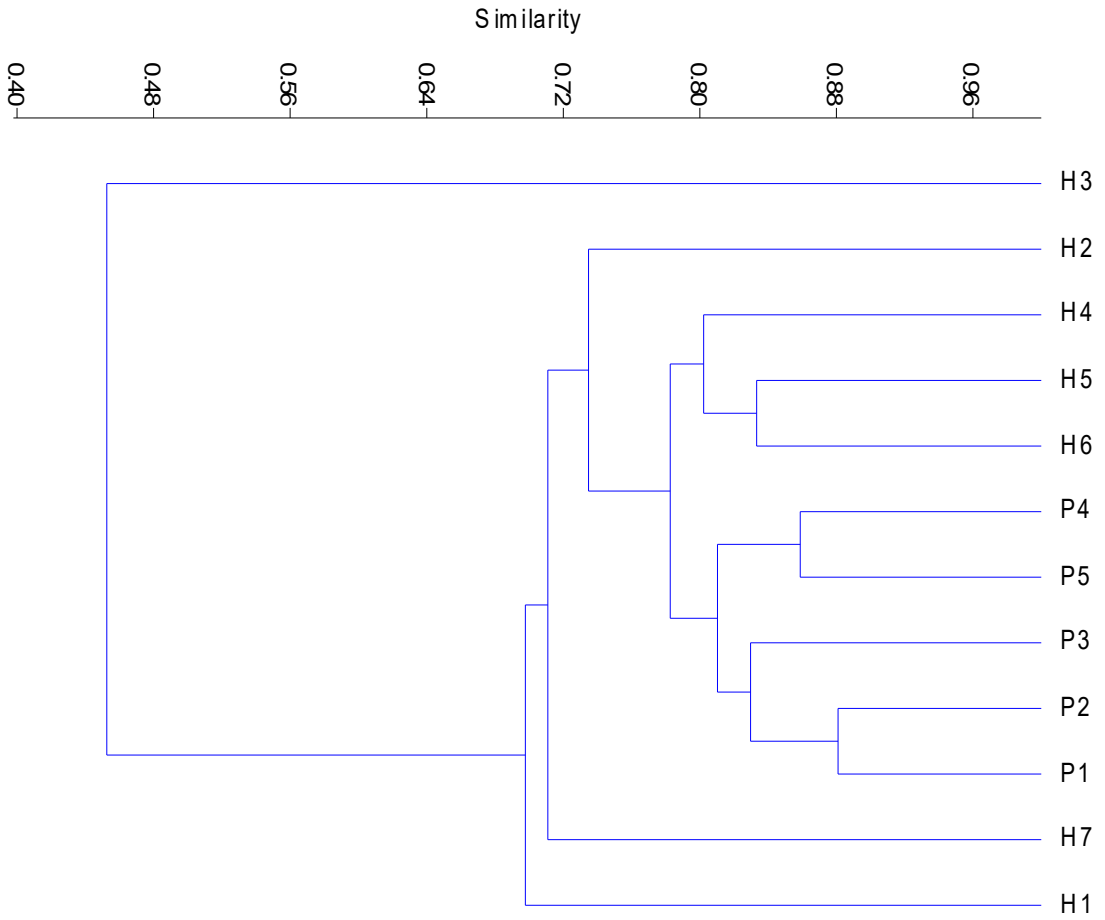

Fig. 2: Dendrogram analysis between 12 Wheat accessions across ISSR markers

4. Discussion

Results shown and viewed in table (5) have proven that the first three wheat parents and the five promising hybrids resulting from it within half diallel analysis respectively (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 and P4 X P5) gave significant and highly significant differences and were various from each other in genetic and phenotypic composition as well, and this qualified them to be fruitful genetic materials in this investigation, table (5). Further, these promising genotypes have actually achieved tolerance to salt stress under salt stress treatment compared to the standard experiment and this has not happened to the rest of the wheat varieties and hybrids determined under the same treatments, table (5). Also, this confirms beyond doubt that these superior entries mentioned above have attributes and mechanisms that carry it at the genetic and physiological levels, which enabled it to endure and resist salt stress. In addition, it was able to excel in the aspect of yield components and physiological traits, such as each both of proline and glycine betaine contents. Further it was able to adjust its osmotic pressure to produce a low osmotic pressure that enables it to endure and survive and give a good crop under conditions of salt stress which known as osmotic adjustment. All of these excellent genetic and biochemical mechanisms were the scientific evidence of salt stress tolerance of these promising wheat accessions. Further, in the end they represent a large and positive physiological leap in this context (El-Mouhamady *et al.,* 2012 a; Ramadan *et al.,* 2016; Esmail *et al.,* 2016; El-Mouhamdy *et al.,* 2016 ; Eldessouky *et al.,* 2016; El-Mouhamady *et al.,* 2022 & El-Mouhamady *et al.,* 2021b). The same five wheat hybrids that were tolerant to salt stress, mentioned above, also proved that they were the highest in the criteria and values of heterosis over better-parent for all attributes studied under both experiments. This is a moral indication of the extent of the extreme genetic isolation that occurred in the first generation hybrid which made them highly tolerant to salt stress, unlike the rest of the genotypes below tested under the same treatments, table (6), (El-Mouhamady *et al.,* 2022 & El-

Mouhamady *et al*., 2021b). Therefore, these promising wheat accessions of first-generation hybrids will be high-yielding lines that are tolerant of environmental stresses, especially salt stress. Also, if these materials are grown for several isolated generations with simple selection to accumulate all the useful quantitative traits and obtain the highest percentage of genetic purity for these new entries. Further, these tolerant hybrids will be an important measure of the SCA effects among the tolerant parents involved in the hybridization, (El-Mouhamady *et al.,* 2022 & El-Mouhamady *et al.,* 2021b). Data viewed in table (7) revealed that the first three wheat entries showed highly significant positively values of GCA effects which confirmed the importance roe of additive gene action for inheriting and controlling salinity tolerance for all attributed under investigation under normal and salinity conditions. This scientific result, of course, confirms the importance of plant genetics and breeding in establishing quantitative traits that are beneficial to plant breeders, such as high yield and tolerance to high salt stress, whether in the soil or in irrigation water. Also, this study has already resulted in the production of three superior wheat hybrids that are highly tolerant to salinity, which qualifies them to be highly output wheat lines that are suitable for resisting environmental stresses in the future, (El-Mouhamady *et al.,* 2014; Al-Kordy *et al.,* 2019 & El-Mouhamady *et al.,* 2021a). Data shown in table (7) revealed that the wheat entries (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 and P4 X P5) were exhibited highly significant values of SCA effects in all traits under study for the two experiments which confirmed the importance role of non- additive gene action for enhancing and inheriting highly yield and its components and physiological attributes besides, increasing the ability of salinity tolerance in wheat accessions under Egyptian conditions. It is worth noting that the measure of SCA effects confirms beyond doubt the importance of plant breeding and the search for useful quantitative traits in this regard. Also, hybrids that excel in high yield and have succeeded in giving high rates in some physiological traits such as proline and glycine betaine contents especially under stress conditions, are definitely tolerant to salt stress. Further, this fact indicates that these new materials are considering the actual nucleus for producing of wheat accessions tolerant to high salinity limit in the future, (El-Mouhamady *et al.,* 2012 c; El-Mouhamady *et al.,* 2014; El-Mouhamady & El-Metwally 2020 & Abdel Sattar & El-Mouhamady 2012). The physiological tests that were conducted by examining the salinity tolerance indices especially for grain yield/plant trait proved beyond a doubt that the five wheat hybrids were purebred which recorded highly rank of yield besides, excelled in all the yield components and physiological traits studied under salt stress experiment compared to the standard treatment were exhibited high level in all salt stress tolerance measurements. Further, these wheat accessions achieved less sensitivity to salt stress and succeeded in reducing the final output. Therefore, this fact is considering a major leap in this regard, table (8), (El-Mouhamady 2023). The plant breeding strategy to confront environmental stresses is a major task that is at the forefront of the priorities of field crop breeding and improvement scientists. Environmental stress, especially salt stress is considering a serious environmental challenge that hinders the crop production process and destroys its final output especially in countries whose national economy depends on agricultural development. Based on this matter, molecular genetics through using molecular markers came as one of the modern branches of pure genetics as an effective means of improving and breeding field crops to confront environmental stresses. Also, molecular markers has proven to be the most successful tool in genetically engineering plants through genetic transfer of tolerance genes responsible for resistant to environmental stresses, such as salt stress, from tolerant wild lines to local accessions that are more sensitive to this dangerous environmental factor, (El-Mouhamady & El-Metwally 2020 & El-Mouhamady 2023). Based on this result, molecular markers has become without a doubt one of the most important ways for engineering, redeveloping and improving crops to obtain high yielding and resistance to abiotic and biotic stresses through genetic engineering and tissue culture programs within the framework of biotechnological technique. This is what we will discuss in detail in this regard. The eleven ISSR markers have proven that they have indeed succeeded in identifying genetic differences at the molecular level between the different wheat genotypes, as they have succeeded in determining which of them are resistant, moderate and sensitive to salt stress in the first F1 generation compared to the original parents in this investigation . Therefore, these genetic indicators will be the main tool for tracking these promising hybrids during their cultivation in segregation generations to obtain new wheat lines of high genetic stability, superior in final output and equally tolerant to salt stress, (El-Mouhamady & El-Metwally 2020 & El-Mouhamady 2023). From another angle, the primers; SR-01, 3, 11 and 45 succeeded in producing and giving the highest rank of amplified fragments (13, 19, 13 & 19) which proved that these markers are

able to identify all genetic differences between parents and new promising wheat hybrids besides, identifying the most important genetic evidence causing salt stress tolerance in this context. Also, SR-01, 3 and 12 primers were generated the highest level of unique markers (5, 10 & 5) in each directions (Positive & negative) among all wheat accessions under study. Thus, these primers are considered tools and molecular genetic indicators to distinguish and determine the best entries in terms of high yield and tolerance to salt stress and an effective means of tracking it through successive segregation generations in the future. This field proves the importance of ISSR markers and their fruitful role in genetic improvement in wheat crops to confront environmental stresses under Egyptian conditions in tables (9 & 10), (El-Mouhamady & El-Metwally 2020 & El-Mouhamady 2023). In the same context, unique markers whether positive or negative, have proven to be a tool for tracking useful quantitative traits during different breeding generations. It has identified accessions that are tolerant to salt stress, whether from parents or hybrids resulting from it, as well as identifying moderately and sensitive genotypes as well. This successful genetic technology has been used for several decades to improve the productivity of a large number of field crops, vegetable and fruit crops, besides, developing horticultural crops in the field of landscaping. As for field crops, biotechnology, especially genetic engineering, the field of genetic transfer, and mutations in the field of tissue culture, have played a major role in bringing about a scientific shift and a major genetic revolution in improving rice crop for water and salt stress tolerance and resist diseases. Also, improving wheat yield for the same scientific purposes mentioned above, especially tolerance to salt stress, (Ramadan *et al.,* 2016 & Esmail *et al.,* 2016). Results shown in table (11) and Fig.2 revealed the importance of genetic similarity and cluster analysis for determining the fruitful role of relationships among all wheat entries under investigating. Further, the degree of similarity, closeness, or genetic divergence between varieties and lines is the basic step in plant breeding programs, especially related to determining the degrees and indications of the ability to combining ability within different genotypes. Also, the process of tracking the genetic relationships that are close together in terms of cultivation, growth and different stages of maturity of hybrids or parents or all wheat genotypes are considering an important classification tool in studying the different paths of genetic stability depending on molecular markers data of ISSR primers. These markers have proven to be capable of determining the mechanisms and characteristics of convergence or divergence between all wheat genotypes mentioned above, as well as determining the mechanisms of salt stress tolerance for hybrids tolerant from the other moderately or sensitive, (Ramadan *et al.,* 2016 & Esmail *et al.,* 2016). The genetic similarity values namely; (P1 & P2) (0.880), (P2 & P5) (0.852), (P2 & H5) (0.822), (P3 & H6) (0.813) and (H2 & H5) (0.818) were the best and optimum relationships among all wheat entries. These results confirmed that the previous genotypes were higher compatibility for growing together for several segregation generations to obtain new whet lines tolerated to salinity besides, its high yielding in this regard in table (11). These promising wheat accessions were consistent not only in agriculture and growth stages, but also in high productivity, disease resistance, and tolerance to unfavorable environmental stresses, most notably salt stress which has become a serious environmental factor that limits crop productivity, especially crops sensitive to it. On this basis, ISSR markers have already succeeded in identifying these successful genetic relationships, and this will help in future studies in selecting the most successful entries to be included in plant breeding programs for improving a very large number of it through hybridization and genetic engineering programs to reach lines characterized by all the quantitative attributes that are sought plant breeder, (Ramadan *et al.,* 2016 & Esmail *et al.,* 2016). Further, the phylogenetic tree succeed in determining all relationships of all wheat entries under study that very related to salinity stress tolerance. Whereas, these genetic relationships mentioned above are the primary turn in determining any of these wheat genotypes, the predecessor of the hoping, suitable for agriculture and growth together from that genetically diverged from each other in (Fig. 2) in order to ultimately obtain genetic and highly improved wheat lines and bearing saline stress as well, their genetic stability which is closing 100%,, (Zian *et al.,* 2013; Ramadan *et al.,* 2016; Esmail *et al.,* 2016; El-Mouhamady *et al.,* 2017; Khatab & El-Mouhamady 2022 & El-Mouhamady 2023). This study has succeeded in identifying all physiological, morphological, biochemical and genetic molecular mechanisms responsible for tolerance to salt stress in some promising wheat genotypes. The strategy of this investigation centered on the actual assessment of tolerance and resistance to environmental stress factors such as salinity, the frequency of which began to gradually increase due to limited irrigation water especially under Egyptian conditions. This remarkable progress confirms beyond doubt the successful integration of plant breeding science, including the transfer of

important quantitative traits through simple hybridization between tolerant lines and accessions to sensitive local varieties, as well as biotechnology, which helps in a way to transfer the superior genes required for tolerance and high yields in a short time. This was a success achieved in this study because ISSR markers showed significant genetic differences between the parents and their hybrids at the partial level. Also, these differences were the genetic evidence of the previous promising hybrids' tolerance of salt stress besides, being tracked across the segregation generations to reach the maximum levels of genetic stable and high yields in the new wheat lines in this regard. On this basis, plant breeding science cannot be separated from biotechnology technique because both of them are complementary to show the true meaning of hybrid vigor and excellent quantitative attributes which is the desired goal for breeders. If the results and outputs of the current study are carefully considered, it can be said that all previous results were promising and highly satisfactory where the plant breeding science especially using the simple hybridization method, has been able to achieve a significant number of important quantitative traits, such as high yield and tolerance to salt stress, from tolerant genotypes to sensitive Egyptian varieties. In the same track, molecular genetics through using ISSR primers were succeeded in determining all genetically differences in the tolerance wheat hybrids than the other sensitive materials under the same conditions. Further, generating 34 unique markers that were be a taxonomic basic in these promising accessions and this a big goal in this investigation.

5. Conclusion

The previous investigation was conducted to study the importance role of physiological, morphological and biochemical mechanisms responsible for salinity stress tolerance in wheat accessions. Where, this strategic crop is considering the great food importance at all levels, whether local or global. Further, the serious scientific attempt to find new wheat entries that are highly tolerant to salinity, high in yield and highly genetically stable in preparation for its cultivation under Egyptian conditions. For this purpose, a selected group of morphological and physiological attributes and salt stress tolerance indices were used, as well, a group of ISSR primers to determine all genetic differences between these superior wheat genotypes and determine the mechanisms of salt stress tolerance at the molecular level. The final results were really promising regarding the existence of five salt-tolerant and high-yielding hybrids besides, highly polymorphism % among them closed to 100.0% using SR-08 and SR-45 primers. All ISSR primers succeed to generate 34 specific markers among all wheat accessions under study as distinct taxonomic markers for genotypes tolerant to salt stress in this regard.

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