

GENETIC EVALUATION OF SOME RICE GENOTYPES WITH THEIR HALF DIALLEL HYBRIDS AND GENETIC VARIATION ASSESSMENT USING ISSR MARKERS UNDER SALINITY STRESS

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ABSTRACT: Salinity is the second main grained challenge facing rice production world wide and Egypt too. Selection of the best genotypes and crosses under salinity condition and integrated it in salinity breeding program to improvement the genetic background is a first demand to increase the yield under this condition. The present investigation was carried out at the experimental farm of El-Sirw Agricultural Research Station, Damietta governorate, Egypt to evaluate some rice genotypes and their crosses of half diallel crosses under saline soil condition during 2021-2022 seasons. Eight varying different rice genotypes are crossed by using half-diallel mating design and 28 crosses were obtained and evaluated under saline soil conditions. The combining ability and heterosis of genotypes and their crosses were assessed for improving and getting the promising salt tolerant genotypes. ISSR marker was used to find out the genetic variation of tested rice genotypes and the results indicated that the ISSR-03 and ISSR-08 markers can be used in salinity breeding program. The obtained results indicated that the two parents IR45427 and AGAMI M1 were the best parents for general combining ability in most studied traits under saline soil conditions. Among the crosses highest mean values were scored by the crosses; Giza177× IR45427, IR45427×AGAMI, AC2882×AGAMI, Sakha 107× AGAMI and Sakha107× IR45427 indicating their superiority under salinity condition and their validation to be used in breeding program for rice salinity tolerance.

Keywords: *Oryza sativa*, Genetic diversity, ISSR marker, Salinity stress.

INTRODUCTION

Rice is very important crop in Egypt and the production of rice yield faced many challenges among them the salinity which affects on about 30-35% of lands especially northern part of where as rice is cultivated and its yield is restricted (Zayed *et al.*, 2019 and Negm *et al.*, 2019). Rice is characterized as a typical glycophytic crop plant due to its salt sensitivity (Zayed *et al.*, 2019). Salinity is a major abiotic constrain faced by farmers in most rice cultivating areas of the world and improving grain yield in rice is the most important breeding objective (Zayed *et al.*, 2017 and Kargbo *et al.*, 2019). Combining ability analysis helps to identify the parents that have good combining ability of traits in the desirable direction for different targeted traits. Identification of parents with good traits and studying its inheritance of

the targeted traits to the progeny in different cross combination is vital to characterize the nature and magnitude of gene effects in the expression of different traits (Zewdu, 2020). Combining ability analysis gives an idea about the relative importance and magnitude of additive and non-additive types of gene action in the expression of the traits (Griffing, 1956). Molecular markers are useful for evaluating genetic diversity in crop germplasm. Several types of cheap and easily usable molecular markers are being used regularly for studying genetic diversity in rice likes inter simple sequence repeat (ISSR) (Alhasnawi *et al.*, 2015). Inter-simple sequence repeats ISSR are a class of molecular markers based on inter-tandem repeats of short DNA sequences. These regions lie within the microsatellite repeats and offer great potential to determine genetic diversity

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compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously (Goodwin *et al.*, 1997). Inter simple sequence repeat (ISSR) polymorphism was used to determine genetic diversity and phylogenetic relationships in rice genotypes (Haritha *et al.*, 2016). In the present study, ISSR markers have been used in determining genetic diversity among studied genotypes. Knowledge of the genetic diversity between genotypes is useful for authentication of rice genotypes under salinity condition. The diversity is important sources for selecting superior, yet genetically divergent parents to optimize genetic variation in subsequent breeding programs. In order to aid in the selection of parents and the decision of breeding strategies for the improving rice salinity tolerance, the goals of this study were to identify the combining ability and genetic variance components for yield and related traits.

MATERIALS AND METHODS

Plant material

This investigation was carried out in the Rice Research and Training Center (RRTC) facilities at Agriculture Research Stations at Sakha (Kafr Elsheikh Governorate) and El-Sirw (Damietta governorate), Egypt, during 2021 and 2022 rice growing seasons to study the inheritance of some morphological, yield and its components under salinity condition. Eight genotypes namely; Giza177, Sakha107, GZ10305-24-1-2-3, AC2882, Suweon392, IR66159-189-5-5-3, IR45427-2B-2-B-1-2 and AGAMI M1 were

chosen from the gene bank at Rice Research and Training Center. These genotypes have a wide range of variation due to their different genetic background in salt tolerance. The origin, type and tolerant to salinity are listed in Table 1 according to (RRTC, 2019).

Filed experiment conditions

The above mentioned eight rice genotypes utilized in this study were grown in three successive sowing dates during 2020 season rice growing with ten days intervals to overcome the difference of heading date among them. Thirty days after sowing, seedlings of each genotype were individually transplanted in the permanent field in three rows, five meters long and 20 × 20 cm apart between plants and rows. A half-diallel cross were carried out among the eight parents at flowering to produce F₁ hybrids seeds. Bulk emasculation method was practiced by using hot water technique according to Jodon, (1938) and modified by Butany, (1961). A total of 28 crosses were made and the hybrid seeds were grown in 2021 rice growing season as F₁ plants on May 1st and plants were transplanted individually at 25 days after sowing in a randomized complete block design (RCBD), with three replications, each replicate consisted of five rows for each parent and F₁ cross. Each row was 5 meters long containing 25 hills with 20 cm between rows. Weeds were chemically controlled by applying two liters Saturn/ Fadden at four days after transplanting. Nitrogen fertilizer was applied at 60 kg N/fed. All the genotypes, eight parents and 28 F₁ crosses were planted under salinity condition.

Table 1: Origin, type, of the eight rice varieties.

Variety	Origin	Type	Salinity tolerance
Giza177	Egypt	Japonica	Sensitive
Sakha107	Egypt	Japonica	Sensitive
GZ10305-24-1-2-3	Egypt	Japonica	Sensitive
AC2882	Egypt	Japonica	Sensitive
Suweon392	South Korea	Indica/Japonica	Moderate
IR66159-189-5-5-3	IRRI	Japonica	Moderate
IR45427-2B-2-B-1-2	IRRI	Indica/Japonica	Tolerant
AGAMI M1	Egypt	Japonica	Tolerant

Soil analysis

The soil samples were taken from the depth of 0-30cm before conducting the experiment at El-Sirw Agriculture Research Station all samples were then air dried and prepared for chemical analysis. The chemical analysis was carried out using the soil extract 1:5 to estimate the soluble anions, cations and total dissolved salts (TDS). The electrical conductivity (EC) was measured in extract of the soil saturated past (Black *et al.*, 1965 and Chapman and Parker, 1961). Some chemical traits of soil of the experimental sites at El-Sirw Agricultural Station in 2021 season are given in Table 2.

Studied traits

Days to heading (days), plant height (cm), panicle length (cm), number of panicles plant⁻¹, number of filled grains panicle⁻¹, number of unfilled grains panicle⁻¹, spikelets sterility percentage, thousand grain weight (g), panicle weight (g), total biomass weight plant⁻¹ (g), Harvest index (HI %) and grain yield plant⁻¹ were estimated.

Statistical Analysis

Analysis of variance and combining ability

The data of both treatments were subjected to Randomized Complete Block Design (RCBD)

according to Snedecor and Cochran (1967). The analysis of variance, Combining ability analysis and genetic component were estimated according to Griffing (1956) method-2, model-1.

The Estimates of heterosis over the better-parents (HBP%):-The heterosis of an individual hybrid for each trait was determined as the increase of the F₁ hybrid mean over either better parent, these proposed by Mather (1949) and Mather and Jinks (1982).

DNA extraction and amplification

Total DNA was extracted from fresh leaves using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions.

ISSR "Inter Sample Sequence Repeat" ISSR-PCR Reactions:

Six ISSR primers were used in the detection of polymorphism (Table 3). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10pcmol), 3 µl template DNA (10ng) and 7 µl dH₂O, according to (Ibrahim *et al.*, 2019).

Table 2: Some chemical and physical analysis of experimental site during 2021 growing season

Samples soil and water	ECedS/m	CO ₃ ⁻	HCO ₃ ⁻	SO ₄ ⁻	Cl ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
Soil (meq l ⁻¹)	9.63	-	4.02	9.25	73.63	8.85	8.52	70.36	1.44
Water (meq l ⁻¹)	1.85	-	5.50	1.13	8.23	3.23	1.69	8.50	0.21
In soil									
pH	O.M.%			SAR	C.E.C.	ESP		Texture	
8.27	1.92			23.87	37.25	78.91		Clay	

Table 3: The list of primers sequence

Primer Name	Sequence
ISSR-03	5'-ACACACACACACACACYT-3'
ISSR-08	5'-AGACAGACAGACAGACGC-3'
ISSR-09	5'-GATAGATAGATAGATAGC-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR-11	5'-ACACACACACACACACYA-3'
ISSR-12	5'-ACACACACACACACACYC-3'

Thermocycling Profile PCR

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 50s, an annealing step at 45°C for 50s, and an elongation step at 72°C for 1min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

Detection of the PCR Products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Data analysis

For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic

tree (dendrogram) was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al.*, 2001). The PIC value for each locus was calculated according to the formula of Milbourne *et al.* (1997). $PIC = \sum (1 - P_i^2) / n$, where P_i is the frequency of the i^{th} allele, n is the number of bands.

RESULTS AND DISCUSSION

Analysis of variance

The analyses of variance for all studied traits under salinity condition are presented in Table 4. Highly significant mean squares were observed for genotypes, parents, crosses and parents vs. crosses for all traits, except for harvest index. High significant differences were detected among genotypes for all studied traits, indicating differences among tested genotypes, since genetic diversity enables the plant breeder to select the best genotypes and integrate them in breeding programs in order to acquire the best promising and high genotypes in the grain yield, and this is the fundamental necessity to complete the study (Zayed *et al.*, 2016 and El-Mowafi *et al.*, 2022). Al-Daej, (2022) study the similar traits and found the mean square among genotypes was extremely significant for all of the analyzed traits.

Table 4a: Mean square estimates of ordinary analysis and combining ability analysis for studied traits under salinity condition.

Sources of variance	d.f	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	No. of Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Replications	2	3.25	10.48	6.38	1.01	37.16	5.65
Genotypes	35	274.43**	407.45**	12.93**	54.21**	1073.24**	120.84**
Parents	7	239.76**	448.57**	7.63**	52.19**	776.18**	167.39**
Crosses	27	239.56**	287.99**	11.30**	47.98**	772.84**	102.23**
P.vs. C.	1	1458.48**	3345.2**	94.00**	236.51**	11263.5**	297.36**
Error	70	12.28	19.18	2.20	6.15	53.18	18.88
GCA	7	330.93**	344.89**	8.35**	37.27**	735.17**	97.15**
SCA	28	31.61**	83.55**	3.30**	13.27**	263.39**	26.06**
Error	70	4.09	6.39	0.73	2.05	17.73	6.29
GCA/SCA		1.19	0.44	0.30	0.31	0.29	0.46

Table 4 b: Mean square estimates of ordinary analysis and combining ability analysis for studied traits under salinity condition.

Sources of variance	d.f	Spikelet's Sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Total Biomass plant ⁻¹ (g)	Harvest index (%)	Grain yield plant ⁻¹ (g)
Replications	2	14.19	2.15	0.011	11.04	30.47	22.66
Genotypes	35	60.38**	3.66**	0.636**	574.81**	71.41**	140.85**
Parents	7	130.94**	3.97**	0.200**	461.26**	205.69**	98.11**
Crosses	27	42.64**	2.86**	0.624**	484.68**	28.15	103.61**
P.vs. C.	1	45.55**	23.20**	4.011**	3803.2**	299.19**	1445.2**
Error	70	7.87	1.03	0.013	12.99	28.05	12.21
σ^2 GCA	7	55.30**	1.67**	0.541**	483.55**	70.64**	123.24**
σ^2 SCA	28	11.33**	1.11**	0.130**	118.62**	12.09	27.88**
Error	70	2.62	0.34	0.004	4.33	9.35	4.07
GCA/SCA		0.60	0.17	0.429	0.42	2.23	0.50

*and **: Significant at 0.05 and 0.01 levels of probability, P.vs. C.: parent vs. crosses

The significant difference of mean squares between parents and crosses indicates that they are suitable for combining ability studies. Moreover, significant mean squares of parent vs. crosses revealed good scope for manifestation of heterosis in all the studied traits. These results agree with those obtained by Zayed *et al.* (2017), Hadifa *et al.* (2018) and El-Mowafi *et al.* (2022) who reported significant differences among genotypes, parents, parents vs. crosses and crosses for agronomic and yield traits.

Both GCA and SCA variances were highly significant for all studied traits except for harvest index the SCA was not significant, indicating the importance of both additive and non-additive genetic variances in determining the inheritance of the studied traits. Similar results were obtained by Zayed *et al.* (2017), Abd El-Aty *et al.* (2022) and El-Gamaal *et al.* (2022). GCA/SCA ratio was more than unity for days to heading and harvest index, these results suggesting the importance of additive type of gene action in the inheritance of this trait. Similarly, Abd El-Aty *et al.* (2022) found GCA/SCA ratio was more than unity for days to heading. On the other hand, GCA/SCA ratio was less than unity for rest of studied traits, indicating that the non-additive type of gene action had greater importance in inheritance of all these traits. GCA/SCA ratio was used to clarify the nature of gene action involved. That's

agree with Farid *et al.* (2016) and Zayed *et al.* (2017) who reported highly significant estimates of both GCA and SCA for all studied traits.

Mean performance

Regarding for days to heading, the genotypes, IR66159×AGAMI, Suweon×AGAMI, IR66159×IR45427, GZ10305×AGAMI, Sakha107×AGAMI, IR45427×AGAMI and AGAMI gave the highest values under salinity condition, while the earlier genotypes were found in the parents, Sakha107 and AC2882 and the crosses, Giza177×GZ10305 and Giza177×Sakha107, accordingly with keeping high yielding the breeders always prefer the short life rice plants which can maintain the water and soil to other using. For plant height, among parents the lowest mean values was observed in IR66159 followed by AC2882 and Sakha107 with (76.67,86.67 and 87.67cm, respectively), while the tallest parent was AGAMI with (117.67cm). Furthermore, the tallest crosses were IR45427×AGAMI (129.33) and Suweon×AGAMI (124.33cm). On the other side, the shortest crosses were Giza177×Sakha107 (90.00) followed by the cross of Giza177×GZ10305 (91.67) without any significant differences. It is considerable to note that most of F₁mean values were directed towards the tall parents, these in turn, suggested that tallness was dominant under saline soil condition (Negm, 2016 and Zayed *et al.*, 2017).

Even though the breeders select the short plants which suitable for mechanical harvesting and less affected by wind, the sensitive plants under salinity are tend to be stunted or short stature, however it must be select other good traits likes grain yield along with plant height if the breeder need to decrease the plant height in rice under salinity condition. In respect to panicle length, the most desirable mean values were obtained from the genotypes, GZ10305×IR45427, Suweon×IR45427 and Giza177×IR45427. On the other hand the lowest values obtained from the parents, Sakha107 and IR66159 and the cross Sakha107×Suweon. These results agree with Zayed *et al.* (2016) and Hadifa *et al.* (2018). Besides, the parents IR45427 followed by AGAMI have the most desirable mean values for number of panicles plant⁻¹, the most desirable mean values were obtained from the crosses, IR45427×AGAMI, Suweon×AGAMI and Giza177×IR45427 under salinity condition. Concerning filled grains panicle⁻¹, among parents IR45427, AGAMI and Suweon392 scored the highest mean, on the other hand, Giza177 had the lowest mean value under salinity condition. Moreover among the crosses the highest mean value were scored by Giza177×IR45427, Sakha107×IR45427, GZ10305×IR45427 and the cross IR45427×AGAMI. Concerning the unfilled grains panicle⁻¹ the parents AGAMI and AC2882 and the crosses AC2882×AGAMI and Giza177×AC2882 gave the lowest mean and most desirable values under salinity condition. For spikelets sterility percentage, among parents the lowest mean value was scored by AGAMI (12.04%) while, the highest mean value was obtained by IR66159 (34.87%). On the other side the crosses, Giza177×AC2882, GZ10305×IR45427 and GZ10305×AGAMI scored the lowest means without any significant differences between them. For thousand grain weight, as in Table 5, among parents, the highest values were scored by Giza177 and AC2882. Among hybrids the highest mean values were scored by the hybrids Sakha107×AC2882, AC2882×IR45427 and Giza177×Sakha107. Regarding panicle weight, the highest mean values were observed for parents IR45427 and AGAMI. On the other side, among hybrids the highest mean values were observed for AC2882×IR45427, Sakha107×IR45427, Giza177×AGAMI, GZ10305×IR45427 and Giza177×IR45427. Similar results

were obtained by Zayed *et al.* (2016), Hadifa *et al.* (2018) and El-Gamaal *et al.* (2022). For total biomass, among parents the genotypes AGAMI followed by IR45427 scored the highest mean. On the contrary, Giza177 and Sakha107 exhibited the lowest values under salinity conditions. Among hybrids the crosses IR45427×AGAMI, Giza177×IR45427 and AC2882×IR45427 scored the highest values for total biomass. Regarding harvest index among parent IR45427 scored the highest mean along with the crosses AC2882×AGAMI, Sakha107×AGAMI, IR66159×IR45427, Sakha107×IR45427 and Giza177×GZ10305 without any significant differences between them. For grain yield plant⁻¹, the results in Table 5 indicated that among parents the highest mean values were had by IR45427 (27.33g) followed by AGAMI (25.54g). Among hybrids the highest mean values were scored by the hybrids Giza177×IR45427 (36.89g), IR45427×AGAMI (34.67g), AC2882×AGAMI (34.33g) and Sakha177×AGAMI (33.44g). Indicating, these parents and crosses could be used in breeding programs to enhance these traits. These results agree with Similar results were obtained by Zayed *et al.* (2016 and 2018), Hadifa *et al.* (2018) and El-Gamaal *et al.* (2022).

Combining ability

General combining ability effects

Theoretically, an estimate of GCA effects of a parent depends upon the group of other parents of which it was crossed with. If the parent is exactly as the overall parental average in its GCA effects, the expected estimate of GCA would be zero. Significant departure from zero either to positive or negative direction would indicate that the parent is much better or much worse than the overall average of the parental lines involved (Farid *et al.*, 2016). Highly significant and positive values of (GCA) would be of interest in some traits such as the traits that have high contribution to yield. However, high negative values for days to heading and plant height would be useful from the breeder's point view of general combining ability effects of each parent for all traits, which are useful for salinity tolerance trait. Combing ability analysis provides guide line for the assessment of relative breeding potential of the parents and help in choice of

parents which may be hybridized either to exploit hybrid vigor by accumulating unfixable gene effects, or to evolve cultivars by accumulating fixable gene effects (Negm, 2016). For days to heading, the results in Table 6 indicated that rice genotypes, Giza177, Sakha107, GZ10305 and AC2882 were the best

parents where they had desirable and highly significant negative GCA effects, while undesirable and highly significant positive GCA estimates were obtained by IR66159, IR45427 and AGAMI. These results agree with Farid *et al.* (2016), Zayed *et al.* (2017) and El-Gamaal *et al.* (2022).

Table 5 a: Mean performance of studied traits of some studied rice genotypes and their crosses under salinity condition.

Traits Genotypes	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	No. of Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Giza177	91.67	89.00	20.00	12.00	66.34	21.44
Giza177×Sakha107	93.00	90.00	20.50	18.00	82.67	27.33
Giza177×GZ10305	91.00	91.67	21.53	24.67	94.11	24.78
Giza177×AC2882	94.67	93.33	22.40	26.33	114.67	17.45
Giza177×Suweon	100.00	100.00	21.10	24.33	107.09	26.74
Giza177×IR66159	107.33	106.67	23.47	18.67	99.10	31.11
Giza177×IR45427	105.00	111.67	24.80	26.33	135.53	34.78
Giza177×AGAMI	118.33	118.33	24.40	23.67	128.90	29.44
Sakha107	87.33	87.67	18.07	13.67	70.11	17.89
Sakha107×GZ10305	101.67	101.67	20.33	21.33	89.67	24.33
Sakha107×AC2882	91.67	98.33	19.37	18.67	100.22	18.44
Sakha107×Suweon	101.67	101.67	19.30	18.00	110.20	28.78
Sakha107×IR66159	108.33	108.33	20.17	18.67	92.67	30.78
Sakha107×IR45427	111.67	110.00	24.00	25.67	129.33	28.00
Sakha107×AGAMI	115.67	113.33	22.57	22.33	116.79	22.89
GZ10305-24-1-2-3	93.33	91.00	18.80	16.33	79.70	20.08
GZ10305×AC2882	96.67	95.00	19.93	13.33	76.00	24.33
GZ10305×Suweon	103.67	105.00	22.30	17.67	101.90	35.22
GZ10305×IR66159	100.67	103.33	22.83	15.67	111.43	38.56
GZ10305×IR45427	104.00	105.00	25.90	15.33	129.32	20.00
GZ10305×AGAMI	116.67	116.67	23.77	17.00	119.85	19.44
AC2882	89.00	86.67	19.17	17.67	82.44	17.15
AC2882×Suweon	101.67	106.67	20.83	23.67	89.88	18.67
AC2882×IR66159	102.33	116.00	24.47	18.67	108.10	26.67
AC2882×IR45427	103.00	117.67	22.93	21.67	125.67	25.08
AC2882×AGAMI	108.00	106.00	21.13	19.67	109.77	15.33
Suweon392	97.33	99.33	21.07	22.00	93.56	22.11
Suweon×IR66159	108.67	98.33	19.37	15.33	95.34	26.33
Suweon×IR45427	108.33	103.33	25.37	25.67	109.47	22.00
Suweon×AGAMI	119.67	124.33	19.97	26.33	93.00	28.67
IR66159-189-5-5-3	105.33	76.67	18.50	13.33	68.33	37.67
IR66159×IR45427	118.00	101.67	20.90	19.67	111.37	32.33
IR66159×AGAMI	124.67	118.33	21.80	21.33	106.43	19.08
IR45427-2B-2-2B-1	100.67	99.67	22.93	22.33	112.80	26.44
IR45427×AGAMI	115.67	129.33	23.43	27.33	138.33	30.42
AGAMI M1	113.67	117.67	20.33	21.33	95.00	13.00
LSD. 0.05	5.72	7.15	2.42	4.05	11.91	7.10
LSD. 0.01	7.61	9.51	3.22	5.39	15.84	9.44

Table 5 b: Mean performance of studied traits of some studied rice genotypes and their crosses under salinity condition.

Genotypes	Traits	Spikelet's Sterility (%)	Thousand grain weight (g)	Panicle weight (g)	Total Biomass plant ⁻¹	Harvest index (%)	Grain yield plant ⁻¹
Giza177		24.11	26.40	1.865	41.00	29.55	12.11
Giza177×Sakha107		24.79	27.50	1.848	45.11	34.93	15.78
Giza177×GZ10305		20.69	26.60	2.050	53.89	38.90	20.95
Giza177×AC2882		13.13	24.97	2.386	70.56	37.03	26.17
Giza177×Suweon		20.65	25.33	2.275	75.67	41.26	31.22
Giza177×IR66159		22.01	26.07	2.311	69.56	35.22	24.44
Giza177×IR45427		20.28	25.67	3.151	91.00	40.34	36.89
Giza177×AGAMI		18.56	24.10	3.176	79.78	42.12	33.44
Sakha107		20.16	25.30	1.797	44.56	30.87	13.73
Sakha107×GZ10305		19.67	26.20	2.049	54.44	34.36	18.67
Sakha107×AC2882		15.54	27.07	2.374	69.33	37.82	26.22
Sakha107×Suweon		20.69	26.50	2.487	72.33	40.37	29.19
Sakha107×IR66159		22.71	25.20	1.718	62.89	36.94	23.22
Sakha107×IR45427		17.87	27.17	3.186	75.56	43.79	33.00
Sakha107×AGAMI		16.35	26.37	2.624	83.78	39.12	32.75
GZ10305-24-1-2-3		20.08	25.63	1.835	42.78	34.82	14.89
GZ10305×AC2882		24.17	27.17	1.923	51.63	34.28	17.68
GZ10305×Suweon		24.45	24.33	2.216	61.78	43.21	26.67
GZ10305×IR66159		25.01	26.17	2.317	48.00	39.82	19.11
GZ10305×IR45427		13.35	27.03	3.172	68.67	42.44	29.11
GZ10305×AGAMI		13.92	24.40	2.894	74.66	42.41	31.67
AC2882		17.26	25.93	1.950	57.40	34.62	17.75
AC2882×Suweon		17.16	25.77	2.104	74.44	38.57	28.78
AC2882×IR66159		19.84	25.83	2.180	53.33	40.68	21.67
AC2882×IR45427		16.69	27.70	3.213	85.22	37.93	32.33
AC2882×AGAMI		12.30	26.33	2.717	80.45	42.94	34.33
Suweon392		19.03	25.03	2.023	49.44	52.28	23.11
Suweon×IR66159		21.65	26.53	1.987	52.11	43.23	22.22
Suweon×IR45427		18.06	25.67	2.932	76.00	43.89	33.33
Suweon×AGAMI		23.76	24.57	2.341	83.67	43.89	31.76
IR66159-189-5-5-3		34.87	24.33	1.980	63.47	26.18	16.50
IR66159×IR45427		22.64	26.77	2.920	64.42	42.56	27.33
IR66159×AGAMI		15.20	26.33	2.661	82.67	38.88	32.14
IR45427-2B-2-2B-1		18.99	23.33	2.356	70.11	42.82	27.33
IR45427×AGAMI		17.98	25.43	2.881	81.00	42.92	34.67
AGAMI M1		12.04	23.33	2.512	71.89	36.78	25.54
LSD. 0.05		4.58	1.66	0.189	5.89	8.65	5.71
LSD. 0.01		6.09	2.20	0.251	7.83	11.50	7.59

*and **: Significant at 0.05 and 0.01 levels of probability, respectively

Table 6 a: Estimates of general combining ability (GCA) effects for studied traits for eight parents under salinity condition.

Genotypes	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Giza177	-4.48**	-4.52**	0.38	0.51	-2.90*	0.88
Sakha107	-3.92**	-3.62**	-1.21*	-1.09	-6.20**	-0.94
GZ10305	-3.65**	-3.45**	-0.02	-2.33**	-4.21**	0.11
AC2882	-6.15**	-2.85*	-0.50	-0.36	-3.46*	-4.54**
Suweon392	0.08	0.32	-0.41	1.41	-2.98*	0.49
IR66159	4.32**	-2.88*	-0.44	-2.63**	-6.27**	5.45**
IR45427	2.95**	4.32**	1.88**	2.54**	18.08**	1.98
AGAMI M1	10.85**	12.68**	0.33	1.94*	7.93**	-3.44**
SE (gi)	0.60	0.75	0.25	0.42	1.25	0.74
LSD. 0.05	1.83	2.05	1.19	1.54	2.64	2.04
LSD. 0.01	2.71	3.03	1.76	2.28	3.90	3.01

Table 6 b: Estimates of general combining ability (GCA) effects for studied traits for eight parents under salinity condition.

Genotypes	Spikelet's Sterility (%)	Thousand grain weight (g)	Panicle weight (g)	Total Biomass	Harvest index (%)	Grain yield plant ⁻¹ (g)
Giza177	1.19	0.10	-0.068	-2.81**	-2.31*	-1.83*
Sakha107	0.15	0.46	-0.172	-4.31**	-2.29*	-2.51*
GZ10305	0.50	0.11	-0.131	-9.70**	-0.69	-3.78**
AC2882	-2.30*	0.47	-0.080	0.41	-1.35	-0.87
Suweon392	0.81	-0.33	-0.121	-0.08	4.70**	1.80
IR66159	4.24**	-0.04	-0.155	-3.57**	-2.23	-2.83**
IR45427	-1.16	0.01	0.457*	8.64**	2.76*	4.99**
AGAMI M1	-3.43**	-0.78	0.272	11.41**	1.39	5.04**
SE (gi)	0.48	0.17	0.020	0.62	0.90	0.60
LSD. 0.05	1.64	0.98	0.332	1.86	2.25	1.83
LSD. 0.01	2.42	1.46	0.491	2.75	3.33	2.70

* and **: Significant at 0.05 and 0.01 levels of probability, respectively.

For plant height, the results in Table 6 indicated that the significant and highly significant negative GCA effects were observed for Giza177, Sakha107, GZ10305, AC2882 and IR66159 those genotypes proved to be good combiners and ascertained their ability of developing short plants, however undesirable and highly significant positive GCA effects were obtained by IR45427 and AGAMI. Concerning panicle length, the rice genotype IR45427 was the best combiner, where it had desirable and highly significant positive GCA effects, on the other side undesirable and significant negative GCA effect was observed for Sakha107. In the

case of panicles plant⁻¹, significant and highly significant positive GCA effects were observed for IR45427 and AGAMI, contrarily highly significant negative GCA effects were exhibited for genotypes GZ10305 and IR66159. Notably, the parents that showed high GCA effects for yield traits also possessed high mean performance of these traits. This displays a favored agreement between the mean performance and GCA effects. Thereby, the parental performance gave a good index for their general combining ability. Similar results were proved by Singh *et al.* (2020) and Rasheed *et al.* (2021). Concerning filled grains panicle⁻¹, highly

significant positive GCA effects were recorded for IR45427 and AGAMI, while the significant and highly significant negative GCA effects were observed for rest genotypes. Moreover, for unfilled grains panicle⁻¹ and spikelets sterility percentage the genotypes, AC2882 and AGAMI showed significant and highly significant negative general combining ability effects, these genotypes could use in breeding program to improve such traits. Similar findings were reported by Zayed *et al.* (2017) and El-Gamaal *et al.* (2022). For thousand grain weight, the best genotypes were Sakha107 and AC2882 which had the positive GCA effects; in addition for panicle weight the best genotypes were IR45427, which had significant positive GCA effects under salinity condition. In the case of harvest index the desirable and highly significant positive GCA effects were observed for the genotypes of Suweon392 and IR45427, these parents could be considered as good general combiners for this trait. Regarding total biomass and grain yield plant⁻¹, results in Table 6 indicated that the genotypes IR45427 and AGAMI were the best combiner due to their highly significant positive GCA effects, so that these parents could be used as a good combiner for improve these traits and could be strongly recommended as good general combiners especially in breeding programs for salinity conditions. All these entries were good combiners for different traits for salt tolerance consequently make breeding program for salinity depended on pyramiding of genes of these traits, and selection must be done in a later generation in order to minimize environmental effects. Negm (2016), Hadifa *et al.* (2018) and El-Gamaal *et al.* (2022) reported that most of the good hybrids were usually generated from the hybrids between parents with high and low GCA. Generally, the highest heterosis was observed in hybrids in which one of the two parents had low general combining ability. This indicated the role of both additive and non-additive gene action in producing heterosis.

Specific combining ability effects

Specific combining ability refers to dominance variance and epistatic interaction (dominance × dominance, additive × dominance or additive × additive). It has relationship with

heterosis, therefore good specific combiners identified in present study for yield and its components are proposed for heterosis breeding. The significance of SCA effects represents dominance and epistatic components of variance (Negm, 2016). Regarding days to heading; desirable significant and highly significant negative SCA effects were exhibited for five crosses i.e. Giza177×Sakha107, Giza177×GZ10305, Sakha107×AC2882, GZ10305×IR66159 and IR45427×AGAMI. Furthermore, for plant height, results in Table 7 showed that the eight crosses; Giza177×Sakha107, Giza177×GZ10305, Giza177×AC2882, GZ10305×AC2882, AC2882×AGAMI, Suweon×IR66159, Suweon×IR45427 and IR66159×IR45427 were detected to exhibit significant and highly significant desirable negative specific combining ability effects since, were the best crosses for this trait. Concerning panicle length, out of 28 hybrids, the desirable and highly significant positive SCA effects were obtained by nine crosses under salinity condition. While, for panicles plant⁻¹, eleven crosses i.e. Giza177×GZ10305, Giza177×AC2882, Giza177×Suweon, Giza177×IR45427, Sakha107×GZ10305, Sakha107×IR66159, Sakha107×IR45427, Suweon×AC2882, Suweon×AGAMI, IR66159×AGAMI and IR45427×AGAMI were the desirable significant and highly significant positive SCA effects (Hadifa *et al.*, 2018 and El-Gamaal *et al.*, 2022). Concerning filled grains panicle⁻¹, the results in Table 7 showed that sixteen crosses out of the 28 hybrids had desirable and highly significant positive SCA effects and five crosses had a highly significant negative SCA effects, showing the major role of environmental variance in the gene expression of this trait. Furthermore, the best crosses which scored highly significant negative SCA estimate for unfilled grains panicle⁻¹ and spikelets sterility percentage were Giza177×AC2882, GZ10305×IR45427, Suweon×IR66159 and IR66159×AGAMI, indicating could use these crosses under salinity condition in breeding program to improving such traits. Vanave *et al.* (2018) study the combining ability under saline soil for similar traits and found similar findings. Regarding thousand grain

weights, the results in Table 7 showed that out of 28 hybrids the significant and positive values of SCA effects were exhibited for two crosses AC2882×IR45427 and IR66159×AGAMI. Hadifa *et al.* (2018) and El-Gamaal *et al.* (2022) were published similar results. In the case of panicle weight, significant positive SCA effects were observed for Giza177×AGAMI,

Sakha107×IR45427, GZ10305×IR45427 and AC2882×IR45427. In the case of total biomass plant⁻¹, highly significant positive SCA effects were exhibited for seventeen hybrids. Concerning to harvest index, desirable significant and highly significant positive SCA effects were exhibited for six hybrids.

Table 7 a: Estimates of specific combining ability (SCA) effects for studied traits of 28 crosses under salinity condition.

Crosses	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Giza177×Sakha107	-2.77*	-5.74**	-0.27	-1.52	-10.87**	2.31
Giza177×GZ10305	-5.03**	-4.24**	-0.42	6.38**	-1.42	-1.30
Giza177×AC2882	1.13	-3.17*	0.92	6.08**	18.39**	-3.97**
Giza177×Suweon	0.23	0.33	-0.47	2.31*	10.33**	0.29
Giza177×IR66159	3.33**	10.20**	1.93*	0.68	5.63**	-0.30
Giza177×IR45427	2.37*	8.00**	0.94	3.18**	17.71**	6.84**
Giza177×AGAMI	7.80**	6.30**	2.09*	1.11	21.23**	6.93**
Sakha107×GZ10305	5.07**	4.86**	-0.04	4.65**	-2.56	0.08
Sakha107×AC2882	-2.43*	0.93	-0.53	0.01	7.24**	-1.16
Sakha107×Suweon	1.33	1.10	-0.69	-2.42*	16.74**	4.14**
Sakha107×IR66159	3.77**	10.96**	0.21	2.28*	2.49	1.19
Sakha107×IR45427	8.47**	5.43**	1.73*	4.11**	14.81**	1.88
Sakha107×AGAMI	4.57**	0.40	1.84*	1.38	12.42**	2.19
GZ10305×AC2882	2.30*	-2.57*	-1.14	-4.09**	-18.97**	3.68*
GZ10305×Suweon	3.07*	4.26**	1.13	-1.52	6.45**	9.54**
GZ10305×IR66159	-4.17**	5.80**	1.70*	0.51	19.27**	7.92**
GZ10305×IR45427	0.53	0.26	2.44**	-4.99**	12.80**	-7.17**
GZ10305×AGAMI	5.30**	3.56*	1.86*	-2.72*	13.49**	-2.30
AC2882×Suweon	3.57**	5.33**	0.14	2.51*	-6.33**	-2.36
AC2882×IR66159	0.00	17.86**	3.81**	1.55	15.18**	0.68
AC2882×IR45427	2.03	12.33**	-0.04	-0.62	8.40**	2.57*
AC2882×AGAMI	-0.87	-7.70**	-0.30	-2.02*	2.65	-1.76
Suweon×IR66159	0.10	-2.97*	-1.39	-3.55**	1.95	-4.69**
Suweon×IR45427	1.13	-5.17**	2.29**	1.61	-8.28**	-5.55**
Suweon×AGAMI	4.57**	7.46**	-1.56*	2.88**	-14.59**	6.54**
IR66159×IR45427	6.57**	-3.64*	-2.14*	-0.35	-3.09	-0.17
IR66159×AGAMI	5.33*	4.66**	0.31	1.91*	2.13	-8.00**
IR45427×AGAMI	-2.30*	8.46**	-0.38	2.75*	9.68**	6.80**
SE (sii)	1.83	2.29	0.78	1.30	3.82	2.28
LSD. 0.05	2.25	2.51	1.46	1.89	3.25	2.51
LSD. 0.01	3.33	3.72	2.17	2.80	4.80	3.71

Table 7 b: Estimates of specific combining ability (SCA) effects for studied traits of 28 crosses under salinity condition.

Crosses	Spikelet's Sterility (%)	Thousand grain weight(g)	Panicle weight (g)	Total Biomass (g)	Harvest index (%)	Grain yield plant ⁻¹ (g)
Giza177×Sakha107	3.84**	1.16	-0.313	-13.96**	0.42	-5.59**
Giza177×GZ10305	-0.60	0.60	-0.151	0.22	2.79*	0.85
Giza177×AC2882	-5.35**	-1.38*	0.134	6.77**	1.57	3.16*
Giza177×Suweon	-0.95	-0.22	0.064	12.37**	-0.24	5.54**
Giza177×IR66159	-3.02**	0.23	0.133	9.76**	0.64	3.39**
Giza177×IR45427	0.64	-0.22	0.362	18.98**	0.78	8.01**
Giza177×AGAMI	1.19	-1.00	0.572*	4.99**	3.93*	4.52**
Sakha107×GZ10305	-0.59	-0.15	-0.048	2.27	-1.77	-0.75
Sakha107×AC2882	-1.91	0.36	0.227	7.05**	2.35	3.89**
Sakha107×Suweon	0.13	0.59	0.380	10.54**	-1.15	4.19**
Sakha107×IR66159	-1.28	-0.99	-0.355	4.59**	2.35	2.85*
Sakha107×IR45427	-0.73	0.92	0.501*	5.04**	4.21**	4.81**
Sakha107×AGAMI	0.02	0.91	0.125	10.49**	0.92	4.51**
GZ10305×AC2882	6.37**	0.80	-0.265	-5.26**	-2.79*	-3.38**
GZ10305×Suweon	3.54**	-1.24*	0.068	5.37**	0.09	2.94*
GZ10305×IR66159	0.67	0.32	0.203	-4.91**	3.62*	0.01
GZ10305×IR45427	-5.59**	1.13	0.446*	3.54**	1.27	2.18
GZ10305×AGAMI	-2.75*	-0.71	0.353	6.77**	2.60	4.69**
AC2882×Suweon	-0.94	-0.16	-0.094	7.93**	-3.89*	2.14
AC2882×IR66159	-1.69	-0.37	0.015	-9.69**	5.15**	-0.34
AC2882×IR45427	0.55	1.44*	0.437*	9.99**	-2.59	2.50*
AC2882×AGAMI	-1.57	0.87	0.125	2.44*	3.80*	4.45**
Suweon×IR66159	-2.99**	1.12	-0.137	-10.42**	1.64	-2.46*
Suweon×IR45427	-1.19	0.20	0.197	1.25	-2.68	0.83
Suweon×AGAMI	6.78**	-0.11	-0.209	6.15**	-1.31	-0.79
IR66159×IR45427	-0.04	1.02	0.218	-6.84**	2.93*	-0.54
IR66159×AGAMI	-5.22**	1.38*	0.144	8.64**	0.61	4.21**
IR45427×AGAMI	2.96*	0.43	-0.247	-5.24**	-0.33	-1.08
SE (sii)	1.47	0.53	0.060	1.89	2.77	1.83
LSD. 0.05	2.01	1.21	0.408	2.28	2.77	2.25
LSD. 0.01	2.98	1.79	0.604	3.38	4.09	3.32

* and **: Significant at 0.05 and 0.01 levels of probability, respectively.

For grain yield plant⁻¹, the results in Table 7 indicated that out of 28 hybrids, desirable significant and highly significant positive SCA effects were observed for fifteen hybrids. From the previous for traits panicle weight, total biomass plant⁻¹, harvest index and grain yield plant⁻¹, the crosses have highly significant positive SCA; Giza177×AGAMI and Sakha107×IR45427 followed by AC2882×IR45427 and AC2882×AGAMI and then IR66159×AGAMI, indicating these crosses the most desirable crosses under salinity condition and could use in breeding program and expect the improvement the grain yield and get promising line by selection in segregating generations. These findings lead to the conclusion that high estimates of specific combining ability effects in any cross combination, might not necessarily be dependent upon the general combining ability effects in the involves parent (Zayed *et al.*, 2017, Hadifa *et al.*, 2018, Vanave *et al.*, 2018 and El-Gamaal *et al.*, 2022).

Estimation of genetic parameters and heritability for studied traits

The estimates of genetic parameters i.e., phenotypic variance (σ^2P), genotypic variance (σ^2G), environmental variance (σ^2E), additive variance (σ^2A), dominance variance (σ^2D), relative importance GCA, relative importance SCA, narrow sense heritability, broad sense heritability and genetic advance (GA %) for all studied traits are presented in Table (8). Additive variance (σ^2A) and relative importance GCA, these results indicated these traits were largely governed by additive gene action, while dominance variance (σ^2D) and relative importance SCA are defined as including the non-additive genetic portion of total genetic variance arising largely from dominance and epistatic deviation. It could be noted that values of additive, dominance and environmental variance varied from traits to another. Selection would be successful during the early generations

when additive gene action is predominant. Otherwise, the selection would be at later generations when these effects are fixed in the homozygous line (Abd El-Aty *et al.*, 2022). Values of additive genetic variance ranged from 0.082 (panicle weight) to 94.36 (filled grains panicle⁻¹). Dominance variance varied from 0.125 (panicle weight) to 245.67 (filled grains panicle⁻¹). For environmental variance, it ranged from 0.004 (panicle weight) to 17.73 (filled grains panicle⁻¹). Also, the highest genotypic variance was recorded for filled grains panicle⁻¹; while, the lowest was estimated for panicle weight. Results of broad sense heritability indicated that all traits have a highest value, except harvest index (%) and thousand grain weight (g). Meanwhile, narrow sense heritability values were low; giving evidence that additive gene action had fewer roles in the genetics of these traits and great role to the other genetic variances. It is of great interest to role that relative importance of SCA was higher than GCA in most traits, indicating that dominance and environmental variance played great role in trait inheritance more than additive gene. In general, normal value of environmental component was estimated for all studied traits but they differ in its magnitude indicating that these traits are affected by the environmental components with different degrees. These findings suggested that the selection during early generation in breeding program could not be implemented under salinity condition, but the selection at late generation has to be practiced under such conditions to minimize environmental effects.

Relative importance of SCA was lower than GCA in, days to heading (days), spikelet's sterility (%) and harvest index (%), indicating that additive gene action played a great role than non-additive gene. On the other hand, the rest traits were governed by dominance gene effects. El-Gamaal *et al.*, 2022) and Abd El-Aty *et al.* (2022)

Table 8 a: Estimates of genetic parameters for studied traits.

Components	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Phenotypic variance (σ^2P)	91.48	135.82	4.31	18.07	357.75	40.28
Genotypic variance (σ^2G)	87.38	129.43	3.58	16.02	340.02	33.99
Environmental variance(σ^2E)	4.09	6.39	0.73	2.05	17.73	6.29
Additive VARIANCE (σ^2A)	59.86	52.27	1.01	4.80	94.36	14.22
Dominance variance (σ^2D)	27.52	77.16	2.57	11.22	245.67	19.77
σ^2A / σ^2D	2.18	0.68	0.39	0.43	0.38	0.72
Relative importance GCA	0.69	0.40	0.28	0.30	0.28	0.42
Relative importance SCA	0.31	0.60	0.72	0.70	0.72	0.58
Narrow sense heritability	0.65	0.38	0.23	0.27	0.26	0.35
broad sense heritability	0.96	0.95	0.83	0.89	0.95	0.84
Genetic advance (GA %)	18.82	22.88	3.55	7.76	37.03	11.03

Table 8 b: Estimates of genetic parameters for studied traits.

Components	Spikelet's Sterility (%)	Thousand grain weight(g)	Panicle weight (g)	Total Biomass	Harvest index (%)	Grain yield plant ⁻¹ (g)
Phenotypic variance (σ^2P)	20.13	1.22	0.212	191.60	23.80	46.95
Genotypic variance (σ^2G)	17.50	0.88	0.208	187.27	14.45	42.88
Environmental variance(σ^2E)	2.62	0.34	0.004	4.33	9.35	4.07
Additive variance (σ^2A)	8.79	0.11	0.082	72.99	11.71	19.07
Dominance variance (σ^2D)	8.71	0.76	0.125	114.29	2.74	23.81
σ^2A / σ^2D	1.01	0.15	0.657	0.64	4.27	0.80
Relative importance GCA	0.51	0.13	0.397	0.39	0.81	0.44
Relative importance SCA	0.50	0.87	0.603	0.61	0.19	0.56
Narrow sense heritability	0.44	0.09	0.39	0.38	0.49	0.41
broad sense heritability	0.87	0.72	0.98	0.98	0.61	0.91
Genetic advance (GA %)	8.04	1.64	0.929	27.87	6.10	12.89

Estimates of better parent heterosis

A large number of crosses exhibited high estimates of heterosis in a desirable direction for different traits under this study. The estimates of heterosis for different traits are presented in Table 9. Heterosis commercialization enhances rice production to cope with continuing global

population growth and future food demand (Huang *et al.*, 2017). For days to heading most of crosses exhibited undesirable significant and highly significant positive heterosis estimate except the Giza177×GZ10305 which have a negative heterosis estimate and a considered a best cross for this trait followed by

Sakha107×AC2882 since it not significant positive heterosis estimate. At the same time, the most crosses exhibited the tallness compared with the shortest parent however there are three crosses, Giza177×Sakha107, Giza177×GZ10305 and Suweon×IR45427 were not significant positive heterosis and it can considered it a best crosses for plant height. A greater magnitude of heterosis ranged between (2.50 and 27.65%) was observed in nineteen crosses for panicle length. Zayed *et al.* (2017) and Thirumalai *et al.* (2017) studied the heterosis over better parents for grain yield panicle length, 100-grain weight. Moreover, highly significant and positive estimates of heterosis were observed for number of panicle⁻¹ in sixteen crosses. Out of 28 crosses nineteen crosses exhibited a desirable significant and highly significant positive heterosis estimates on the other hand only one cross Suweon×IR45427 exhibited a desirable negative heterosis estimated value but not significant for unfilled grain panicle⁻¹. For spikletes sterility percentage, the best crosses were Giza177×AC2882, Giza177×IR66159, Sakha107×AC2882, Sakha107×IR45427, GZ10305×IR45427 and Suweon×IR45427 which have a significant and highly significant negative heterosis estimate and could use in salinity breeding program to improve such trait. Fifteen crosses out of 28 crosses exhibited willingness to improvement in thousand grain weight under salinity stress. For panicle weight, the vigor based on heterosis over better parent represented in twenty-three crosses out of 28 studied crosses which expected improvement in this trait under salinity program using these crosses. For total biomass plant⁻¹, nineteen crosses out of 28 crosses were significant and highly significant positive heterosis estimate. Furthermore, ten crosses were significant and highly significant positive heterosis estimate for harvest index. At

the same time, four crosses Viz; Giza177×GZ10305, Giza177×IR66159, Giza177×AGAMI and AC2882×AGAMI exhibited the highly significant positive for the total biomass, harvest index and grain yield and they could be used in breeding program under salinity condition.

A greater magnitude of heterosis ranged between (6.50 and 48.14%) was observed in twenty-five crosses for grain yield plant⁻¹ indicating the availability of sufficient hybrid vigor in several crosses. Regarding grain yield suggested that a hybrid breeding program could profitably be undertaken in rice under salinity condition (Zayed *et al.*, 2017, El- Mowafi *et al.*, 2022 and Abd El-Aty *et al.*, 2022).

Molecular marker (ISSR-PCR)

The PCR amplification of eight genotypes using six primers, a total of 53 DNA bands was detected for ISSR-PCR analysis (Fig.1 and Table 10), 47 bands were bands, and 14 bands were monomorphic. Furthermore, the unique band was appeared in five from six primers, where ISSR-08 have two unique bands and ISSR-12 didn't have while the rest primer has only one unique band. Unique DNA fragments with different sizes were detected in particular genotype but not in the others using different primers. The presence of a unique band for a given genotypes is referred as positive marker, while the absence of common bands served as negative marker. Such bands could be used as DNA markers for genotype identification and discrimination. In this respect, three DNA unique bands were detected in the variety Giza177, two bands in Sakha107 and one band in AC2882. Therefore, based on the molecular results in this study could be considers that the unique band could be used as a positive marker under salinity condition.

Table 9 a: Estimates of percentage of heterosis over better-parent for the studied traits of rice crosses.

Crosses	Days to Heading (days)	Plant height (cm)	Panicle length (cm)	Panicles plant ⁻¹	Filled grains panicle ⁻¹	Unfilled grains panicle ⁻¹
Giza177×Sakha107	6.49*	2.66	2.50*	31.71**	17.91**	52.79**
Giza177×GZ10305	-0.73	3.00	7.67**	51.02**	18.08**	23.41**
Giza177×AC2882	6.37*	7.69*	12.00**	49.06**	39.09**	1.71
Giza177×Suweon	9.09**	12.36**	0.16	10.61**	14.47*	24.70**
Giza177×IR66159	17.09**	39.13**	17.33**	40.00**	45.02**	45.08**
Giza177×IR45427	14.55**	25.47**	8.14**	17.91**	20.15**	62.18**
Giza177×AGAMI	29.09**	32.96**	20.00**	10.94**	35.68**	126.49**
Sakha107×GZ10305	16.41**	15.97**	8.16**	30.61**	12.51*	36.02**
Sakha107×AC2882	4.96	13.46**	1.04	5.66**	21.57**	7.53*
Sakha107×Suweon	16.41**	15.97**	-8.39**	-18.18**	17.79**	60.86**
Sakha107×IR66159	24.05**	41.30**	9.01**	36.59**	32.17**	72.04**
Sakha107×IR45427	27.86**	25.48**	4.65**	14.93**	14.66*	56.53**
Sakha107×AGAMI	32.44**	29.28**	10.98**	4.69*	22.94**	76.07**
GZ10305×AC2882	8.61**	9.62**	4.00**	-24.53**	-7.82	41.86**
GZ10305×Suweon	11.07**	15.38**	5.85**	-19.70**	8.92	75.44**
GZ10305×IR66159	7.86**	34.78**	21.45**	-4.08*	39.82**	92.05**
GZ10305×IR45427	11.43**	15.38**	12.94**	-31.34**	14.64*	-0.38
GZ10305×AGAMI	25.00**	28.21**	16.89**	-20.31**	26.16**	49.57**
AC2882×Suweon	14.23**	23.08**	-1.11	7.58**	-3.93	8.82*
AC2882×IR66159	14.98**	51.30**	27.65**	5.66**	31.12**	55.46**
AC2882×IR45427	15.73**	35.77**	0.00	-2.99	11.41	46.23**
AC2882×AGAMI	21.35**	22.31**	3.93**	-7.81**	15.54**	17.95**
Suweon×IR66159	11.64**	28.26**	-8.07**	-30.30**	1.91	19.10**
Suweon×IR45427	11.30**	4.03	10.61**	14.93**	-2.96	-0.50
Suweon×AGAMI	22.95**	25.17**	-5.22**	19.70**	-2.11	120.51**
IR66159×IR45427	17.22**	32.61**	-8.87**	-11.94**	-1.27	22.27**
IR66159×AGAMI	18.35**	54.35**	7.21**	0.00	12.04*	46.79**
IR45427×AGAMI	14.90**	29.77**	2.18	22.39**	22.64**	134.00**
LSD. 0.05	5.61	7.01	2.37	3.97	11.67	6.95
LSD. 0.01	7.37	9.21	3.12	5.22	15.34	9.14

Table 9 b: Estimates of percentage of heterosis over better-parent for the studied traits of rice crosses.

Crosses	Spikelet's Sterility (%)	Thousand grain weight(g)	Panicle weight (g)	Total Biomass	Harvest index (%)	Grain yield plant ⁻¹ (g)
Giza177×Sakha107	22.96**	4.17**	-0.91**	1.24	13.14**	14.88**
Giza177×GZ10305	3.06	0.76	9.94**	25.98**	11.70**	40.75**
Giza177×AC2882	-23.91**	-5.43**	22.34**	22.93**	6.97	47.42**
Giza177×Suweon	8.49**	-4.04**	12.44**	53.04**	-21.08**	35.11**
Giza177×IR66159	-8.70**	-1.26	16.72**	9.59**	19.18**	48.14**
Giza177×IR45427	6.81**	-2.78**	33.72**	29.80**	-5.81	34.95**
Giza177×AGAMI	54.13**	-8.71**	26.42**	10.97**	14.52**	30.94**
Sakha107×GZ10305	-2.03	2.21**	11.68**	22.19**	-1.33	25.39**
Sakha107×AC2882	-9.96**	4.37**	21.76**	20.80**	9.26*	47.72**
Sakha107×Suweon	8.68**	4.74**	22.92**	46.30**	-22.79**	26.29**
Sakha107×IR66159	12.67**	-0.40	-13.22**	-0.92	19.65**	40.73**
Sakha107×IR45427	-5.87*	7.38**	35.20**	7.77**	2.25	20.73**
Sakha107×AGAMI	35.81**	4.22**	4.46**	16.53**	6.38	28.23**
GZ10305×AC2882	40.01**	4.76**	-1.38**	-10.04**	-1.57	-0.38
GZ10305×Suweon	28.46**	-5.07**	9.52**	24.94**	-17.35**	15.39**
GZ10305×IR66159	24.56**	2.08*	17.00**	-24.37**	14.34**	15.84**
GZ10305×IR45427	-29.68**	5.46**	34.60**	-2.06	-0.89	6.50*
GZ10305×AGAMI	15.62**	-4.81**	15.19**	3.86	15.30**	23.99**
AC2882×Suweon	-0.57	-0.64	4.02**	29.70**	-26.23**	24.52**
AC2882×IR66159	14.96**	-0.39	10.10**	-15.98**	17.53**	22.07**
AC2882×IR45427	-3.31	6.81**	36.37**	21.56**	-11.44**	18.29**
AC2882×AGAMI	2.17	1.54	8.15**	11.90**	16.77**	34.43**
Suweon×IR66159	13.77**	5.99**	-1.80**	-17.90**	-17.32**	-3.84
Suweon×IR45427	-4.86*	2.53**	24.44**	8.40**	-16.06**	21.95**
Suweon×AGAMI	97.34**	-1.86*	-6.81**	16.38**	-16.05**	24.34**
IR66159×IR45427	19.27**	10.00**	23.92**	-8.12**	-0.61	0.00
IR66159×AGAMI	26.21**	8.22**	5.93**	14.99**	5.72	25.84**
IR45427×AGAMI	49.32**	9.00**	14.69**	12.67**	0.23	26.83**
LSD. 0.05	4.49	1.62	0.18	5.77	8.48	5.59
LSD. 0.01	5.90	2.13	0.24	7.58	11.14	7.35

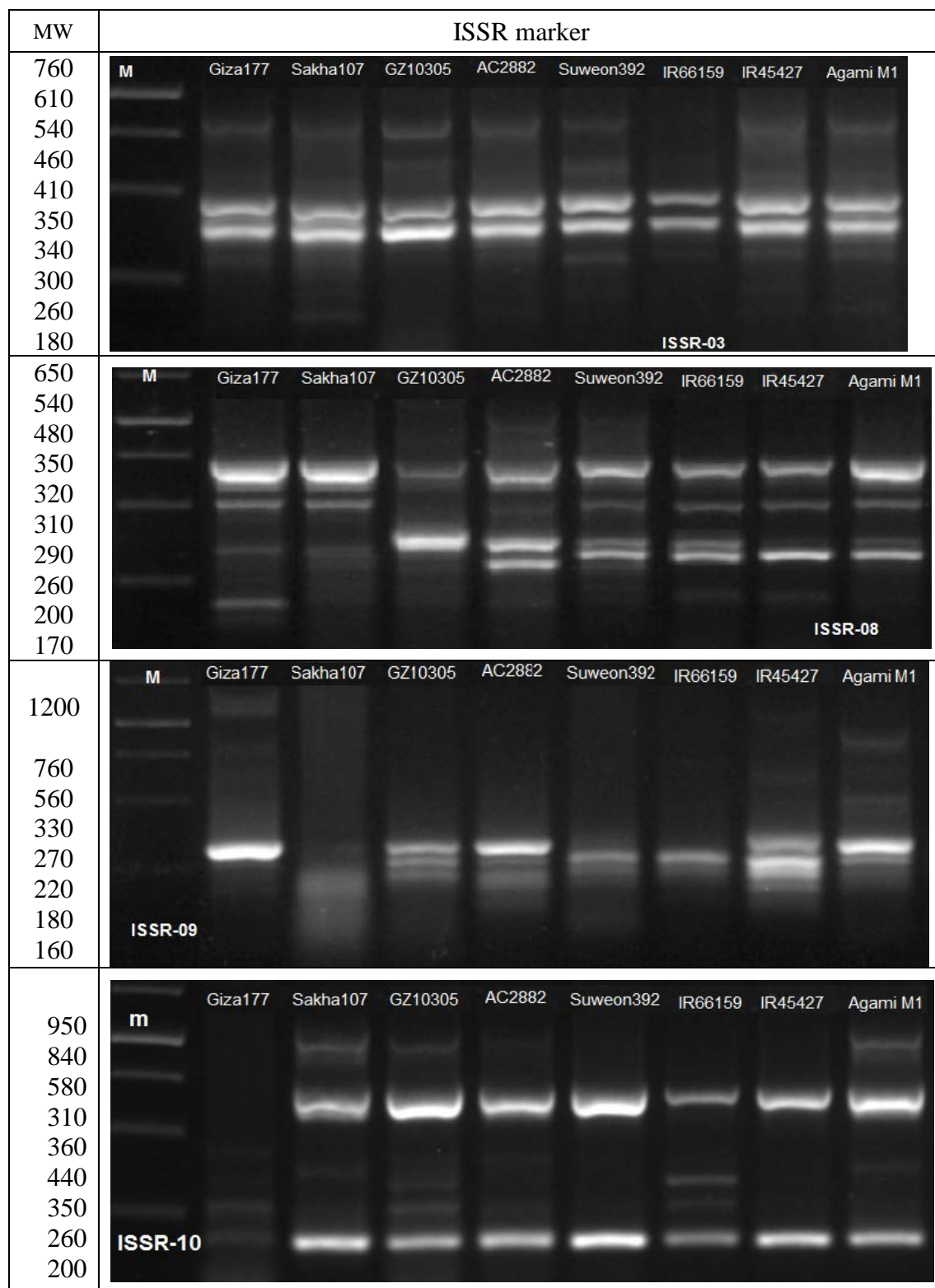


Fig. (1): ISSR profiles , the PCR patterns of the eight rice genotypes using the six ISSR Primers.

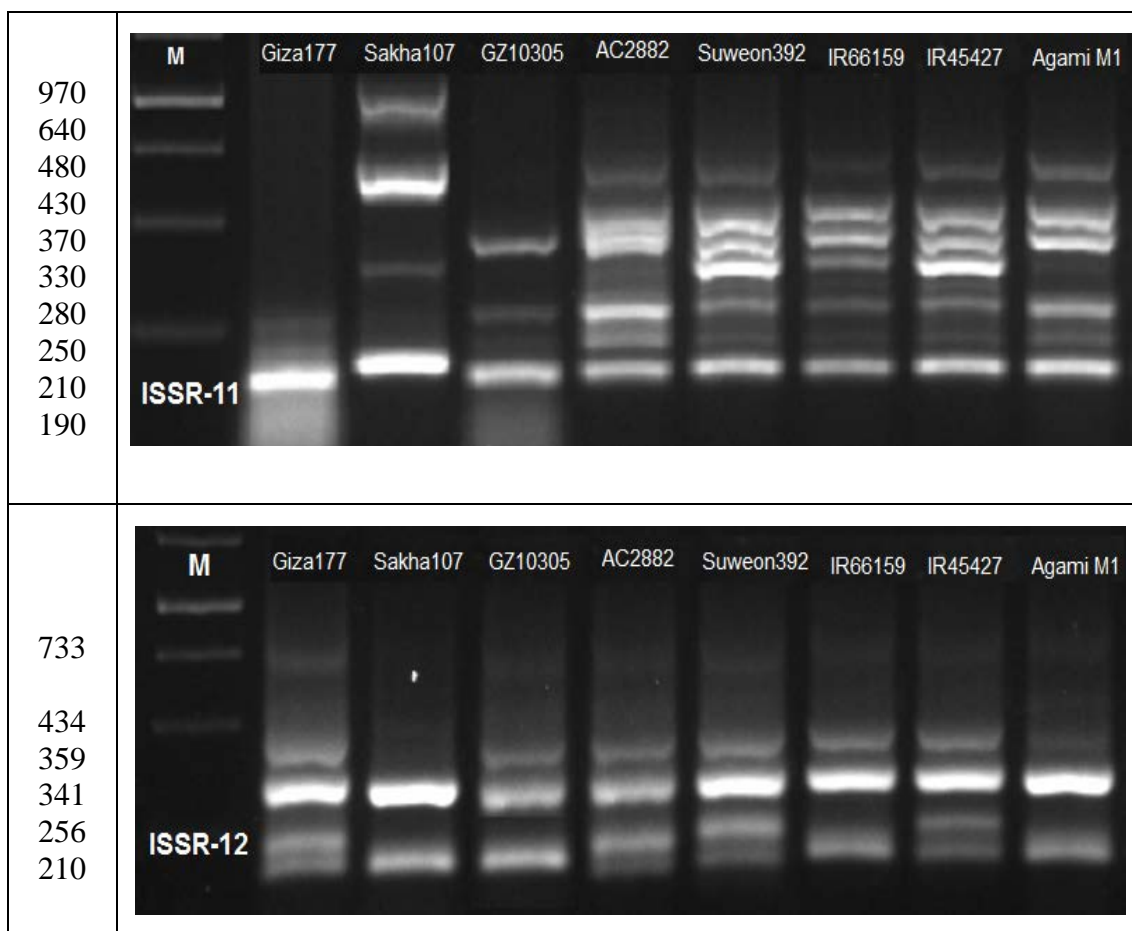


Fig. (1): ISSR profiles , the PCR patterns of the eight rice genotypes using the six ISSR Primers.

Table 10. The banding pattern and polymorphism generated by the six ISSR primers for the eight rice genotypes.

Primer	ISSR-03	ISSR-08	ISSR-09	ISSR-10	ISSR-11	ISSR-12
Monomorphic bands	5	1	0	2	3	3
Polymorphic (without Unique)	4	7	7	6	6	3
Unique bands	1	2	1	1	1	0
Polymorphic (with Unique)	5	9	8	7	7	3
Total number of bands	10	10	8	9	10	6
Polymorphism (%)	50%	90%	100%	78%	70%	50%
Mean of band frequency	0.68	0.55	0.35	0.56	0.69	0.85
Polymorphism information Content. (PIC)	0.42	0.60	0.75	0.60	0.44	0.22

To assess the degree of polymorphism in the tested eight rice parents, the number of polymorphic bands ranged from 50% to 100%. Among six primers the ISSR-09 was showed 100% polymorphism followed by ISSR-08 with 90% polymorphism. The Primer ISSR-12 gave the smallest number of bands, while the highest number of bands was ten bands in ISSR-03, ISSR-08 and ISSR-11 indicating that capability of these primers to distinguish among the studied genotypes. The PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker (Nagy *et al.*, 2012). As it shown in Table 10 the PIC values for the ISSR used in this study varied from 0.22 to 0.80 with an average of 0.75. According to Anderson *et al.* (1993), there were three highly informative markers ($PIC > 0.50$), two informative markers ($0.50 < PIC < 0.25$) and one marker slightly informative markers ($PIC < 0.25$). The highest PIC values were observed for ISSR-09 (0.75). The similarity matrix showed that the highest similarity percentage was obtained with 0.88 between IR45427 and IR66159 genotypes, while the lowest similarity percentage was obtained with 0.60 between the Giza177 and Sakha107 (Table 11). A dendrogram was developed using (UPGMA) and specifically discriminated among the genotypes of rice (Fig. 2).

Cluster analysis based on ISSR marker

The genetic relationships among rice genotypes are presented in a dendrogram based on ISSR data analysis (Fig. 2). The obtained dendrogram showed two main clusters; the first main cluster has only one variety Sakha107. The second main cluster has divided into two sub clusters; one sub-cluster contained one rice variety (Giza177), while the other sub-cluster contained two groups, the first group contain two promising line GZ10305 and AC2882 while the second group contain the rest genotypes where AGAMI M1 listed in separated branch and the second branch contain IR45427 and IR66159 with the same similarity and also this branch contains Suweon 392. These results indicated that the ISSR markers can classified and differentiate between rice genotype based on genetic resources and the tolerant to salinity, which listed the sensitive variety Giza 177 and Sakha 107 in separated cluster and also grouped the tolerant variety AGAMI M1 and IR45427 in the same cluster. These results agree with Reddy *et al.* (2009) and Kumari *et al.* (2020). Furthermore, a similar result was also reported that ISSR markers are used to examine the genetic diversity and relationships between eight Egyptian wheat cultivars (Abdel-Lateif and Hewedy, 2018).

Table (11): Similarity matrix among eight rice genotypes according to Dice coefficient as revealed by ISSR markers.

Genotypes	Giza177	Sakha107	GZ10305	AC2882	Suweon392	IR66159	IR45427
Sakha107	0.60	1.00					
GZ10305	0.72	0.68	1.00				
AC2882	0.67	0.72	0.86	1.00			
Suweon392	0.66	0.68	0.76	0.79	1.00		
IR66159	0.74	0.67	0.79	0.79	0.84	1.00	
IR45427	0.73	0.69	0.74	0.80	0.85	0.88	1.00
AGAMI M1	0.71	0.73	0.78	0.84	0.81	0.83	0.84

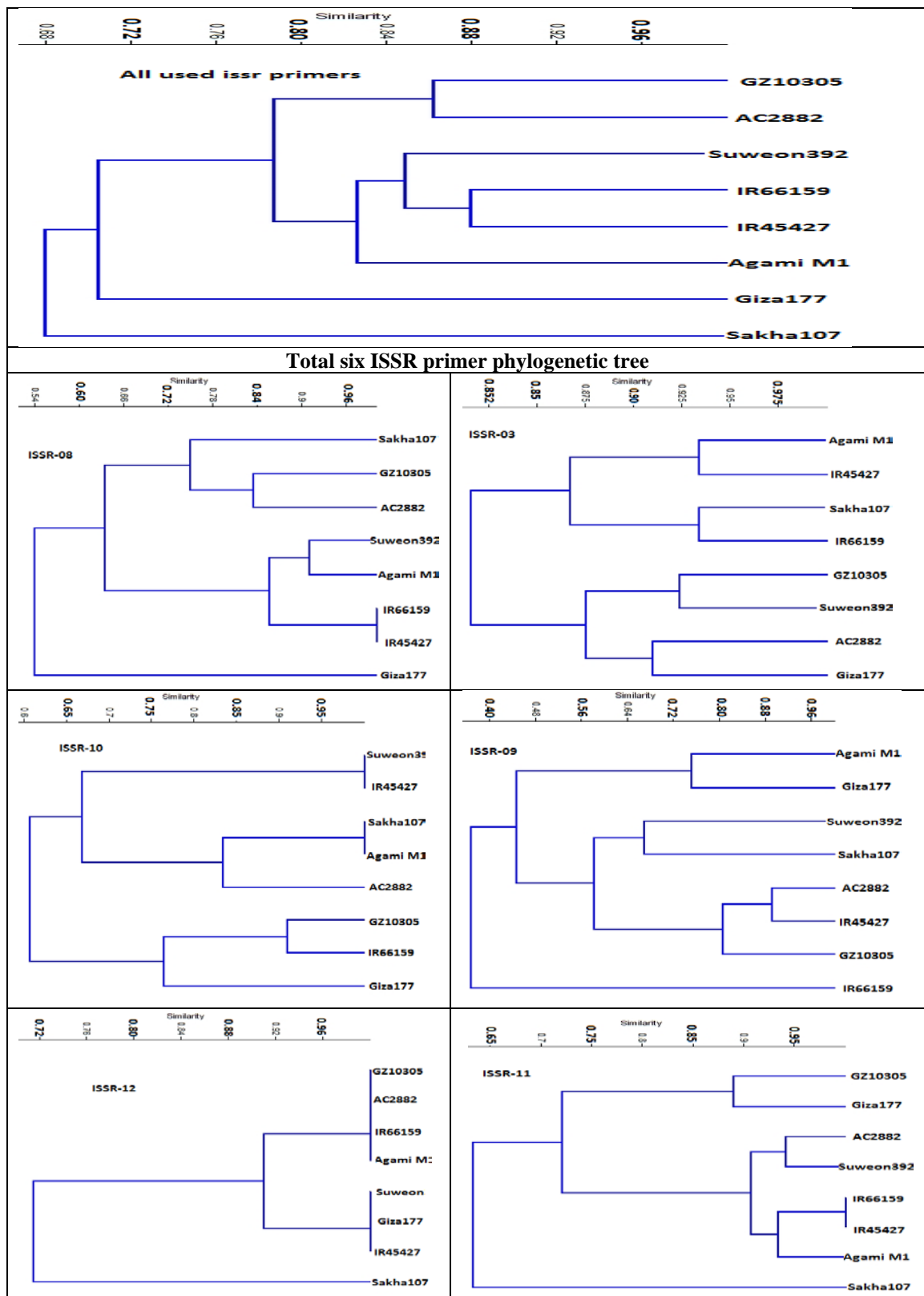


Fig. (2): Dendrogram for the eight rice genotypes constructed from ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

In an attempt to know the best used primer, which can be used to distinguish between cultivars on the basis of tolerance to salinity with linking them to the studied morphological characteristics, which can be used in breeding programs to select the tolerant varieties to salinity in the subsequent study. Among the dendrograms for each primer separately is clarify and the morphological traits, the result indicated that ISSR-03 can be used in breeding program to select the tolerant varieties. This marker classified the tolerant varieties AGAMI M1 and IR45427 in the same group and the rest genotypes in the other group and cluster. Furthermore, the dendrogram based on ISSR-08 showed the Giza177 listed in separated cluster and the variety Giza177 with two promising line GZ10305 and AC2882 listed in the same group, while the foreign genotype listed in the same group with the tolerant variety AGAMI M1 and also could be used in breeding program to distinguish between the tolerant and sensitive variety. This research has demonstrated reliable diversity sources that will assist breeders in identifying genetic variation and selecting economically valuable traits like salinity tolerance. The similar conclusion was drawn from earlier investigations, demonstrating the significance of ISSR markers in the identification of polymorphisms and the formation of particular genetic relationships (Etminan *et al.*, 2016 and El-Saber, 2021). The rest of ISSR markers in this study confirmed the variation between using genotypes and it can lead to good heterosis among these genotypes when crossing it's in breeding program but not clearly classified among genotype based on their salinity tolerance. Analysis of DNA using ISSR-PCR has proven to be excellent markers for providing molecular data to test genetic variation and has been successfully used to classify genetic relationships for many other plants. El-Saber, (2021). Abdi *et al.* (2012) and Kumbhar *et al.* (2013) used ISSR marker to evaluate genetic diversity under salinity condition in cotton and rice, respectively.

CONCLUSION

For forecasting the most tolerant cultivars, breeding programs may greatly benefit from the specific positive marker identification (ISSR-03 and ISSR-08). Specific salinity tolerance markers

can be found using ISSR approaches, but further experimental research is required to determine how these ISSR markers relate to the salt tolerance gene(s) that underlie these rice genotypes. The obtained results indicated that the two parents; IR45427 and AGAMIM1 and the crosses; Giza177×IR45427, IR45427×AGAMI, AC2882×AGAMIS akha177×AGAMI and Sakha107×IR45427 were the most desirable genotypes under salinity condition and they could be used in breeding program for improving the grain yield and getting promising salt tolerant lines.

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التقييم الوراثي لبعض التراكيب الوراثية للأرز وهجنها الناتجة عن طريق التهجين النصف دائري وتقدير التباين الوراثي باستخدام الدليل الوراثي ISSR تحت ظروف الاراضى الملحية

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^(٢) قسم الوراثة ، كلية الزراعة ، جامعة المنوفية ، مصر

الملخص العربى

الملوحة هي ثاني تحد رئيسي يواجه إنتاج الأرز في جميع أنحاء العالم اختيار أفضل التراكيب الوراثية الهجن تحت ظروف الملوحة ودمجها في برنامج تربية الملوحة لتحسين الخلفية الوراثية هو مطلب أول لزيادة المحصول في ظل هذه الظروف. تم إجراء هذا البحث بالمزرعة التجريبية بمحطة بحوث السرو الزراعية بمحافظة دمياط ، مصر لتقييم بعض التراكيب الوراثية للأرز وهجنها تحت ظروف التربة المالحة خلال مواسم ٢٠٢١-٢٠٢٢. تم تهجين ثمانية تراكيب وراثية مختلفة من الأرز باستخدام تصميم تزاوج نصف دائري وتم الحصول على ٢٨ هجين وتقييمها تحت ظروف التربة المالحة. تم تقييم القدرة على التألف بين التراكيب الوراثية وكذلك قوه الهجين لمعرفه والحصول على التراكيب الوراثية الواعدة التي تتحمل الملوحة. تم استخدام الدلائل الوراثية ISSR لمعرفة الاختلاف الجيني لتراكيب الارز الوراثية المختبرة ، وأشارت النتائج إلى أنه يمكن استخدام ISSR-03 و ISSR-08 في برنامج تربية الملوحة. أشارت النتائج المتحصل عليها إلى أن الأبوين IR45427 و AGAMI M1 كانا أفضل الأباء من حيث القدرة على الجمع العام في معظم الصفات المدروسة تحت ظروف التربة المالحة. من بين أعلى القيم المتوسطة تم تسجيلها من خلال الهجن: IR45427 × Giza177 ، IR45427 × AGAMI ، AC2882 × AGAMI ، Sakha107 × IR45427 و Sakha107 × AGAMI مما يدل على تفوقها تحت ظروف الملوحة صلاحيته للاستخدام في برنامج التربية لتحمل الملوحة في الأرز.

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