

## GENETIC AND MOLECULAR STUDIES ON SOME TRAITS CORRESPONDING TO SALINITY TOLERANCE IN RICE

Negm, M.E.; Hadifa, A.A.; Soltan, S.A. and Bassiouni, S.M.

Rice Research department, Field Crops Research Institute, Agricultural Research Center, Egypt

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**ABSTRACT:** The present investigation was carried out at Sakha Agricultural Research Station, Kafr El-Sheikh and clayey saline soil at the experimental farm of El-Sirw Agricultural Research Station, Agriculture Research Center, Damietta governorate (31°14'N and 31°48'E) at the northern east of the Delta, Egypt during 2021 and 2022 seasons. Four Egyptian rice genotypes *i.e.* Giza178, Giza179, GZ9399-4-1-1-3-2-2 as a salt tolerant varieties and Sakha 106 as salt sensitive one were used as line and three rice genotypes; IRR147 and IR65483 (salt tolerant varieties), Egyptian Jasmine ( salt moderately salinity tolerant variety) as tester were utilized. The parental varieties were crossed by using line & tester's analysis and twelve crosses were obtained. The F<sub>1</sub> and their parents were grown and evaluated under saline soil. The studied characters were yield and yield attributes. The obtained results indicated that the parents, Giza178, Giza179 and IRR147 were the best parents for general combining ability for studied traits under saline soil conditions. Meanwhile, the crosses, Giza179×IRR147, Giza178×IRR147 and Giza179×E.Jasmine were the best performing crosses and could be used under salinity condition as well as are considered as the most effective for getting promising lines. Assessing the genetic distance and linkages between seven different rice types were used to perform ISSR markers to identify genetic differences that could aid in the creation hybridization and selection of breeding program.

**Key words:** Rice, Salinity, ISSR markers, general and specific combining ability, genetic parameters.

### INTRODUCTION

Rice (*Oryza sativa* L.) is a very essential cereal crop of the world, grown in wide scope of climatic zones. It is a staple food for nearly half of the world's population, mostly living in developing countries. The crop occupies one third of the world's total area planted to cereals and provides 35 to 60% of the calories consumed by 2.7 billion people. (Mandana *et al.*, 2014). In Egypt, rice ranks the second most important food crop next to wheat and its cultivation mainly is in the north Delta. The soils of north Delta are characterized by high salinity beside its importance as a staple food crop; rice is cultivated as a reclaiming crop for such saline soils (Zayed *et al.*, 2017). Salinity is one of the most important environmental stresses that negatively affect plant productivity its impairment of plant growth and development through water stress, toxicity of ions, nutritional imbalances and oxidative stress (Negm *et al.*, 2019). Soil salinity is one of the major constraints affecting rice production worldwide, especially in the coastal areas, Salt tolerance is

an important constrain for rice (Zayed *et al.*, 2023a). Understanding the genetic behavior of salinity tolerance is a major concern to produce new genotypes suitable for the salt affected areas in Egypt. Molecular markers offer specific advantages in assessment of genetic diversity and in trait specific crop improvement. Use of markers in applied breeding programs can range from facilitating appropriate choice of parents for crosses, to mapping/tagging of gene blocks associated with economically important traits (Farid *et al.*, 2016). Accordingly, the present investigation aimed to study the performance of some rice varieties and their hybrids under saline soil condition and estimate the genetic parameters, combining ability, heritability and heterosis, as well as, assessment the genetic diversity among rice varieties using ISSR markers.

### MATERIAL AND METHODS

The present study was carried out during the summer seasons of 2021 and 2022 at Rice Research department, Sakha, Kafr El-Sheikh and

the experimental farm at El-Sirw Agricultural Research Station, Damietta governorate. Seven genetically diverse rice varieties were chosen on the bases of their salinity tolerance. Four varieties, namely, Giza178, Giza179, GZ9399-4-1-1-3-2-2 and Sakha106 were used as lines, while the three varieties i.e., IRRI147, IR65483-141-2-4-4-2-5 and Egyptian Jasmine were used as testers. A brief summary of the parentage, type and salinity tolerance of these varieties are presented in Table 1.

In 2021 season, seeds of parental genotypes were sown in the green house nursery in three successive sowing dates at seven days intervals to synchronize the flowering of the genotypes. After 25 days, seedlings were transplanted in the experimental field and at heading; the seven parental genotypes were crossed in a Line × Tester set producing twelve crosses. (Kempthorne, 1957). In 2022 seasons, seeds of seven parents and their possible twelve crosses were sown in the nursery and seedlings were

transplanted after 30 days from sowing in a Randomized Complete Block Design experiment (RCBD), with three replications. Each cross consisted of three rows for each parent and F<sub>1</sub>; the row was five meters length with 20 cm. between rows and hills. All recommended agriculture practices for growing rice production in the saline effected soil were applied.

### Soil analysis

Before conducting the experiment, soil samples were taken from depth 0-30cm, all samples were then air dried and prepared for chemical analysis. The chemical analysis was carried out using the soil water extraction 1:5 to estimate the soluble anions and cations. The electrical conductivity (EC) was measured according to Black *et al.*, (1965) and the method of Chapman and Pratt, (1961). Some chemical characters of soil and irrigation water of the experimental site at El-Sirw location in 2022 season are given in Table 2.

**Table 1: Parentage, type and salinity tolerance of parents were utilized in the study.**

Variety	Parentage	Type	Salinity tolerance
Giza178	Giza175/Milyang49	Indica/Japonica	Tolerant
Giza179	GZ1368/GZ6296	Indica/Japonica	Tolerant
GZ9399-4-1-1-3-2-2	Giza178xIR65844-29-1-3-1-2	Indica/Japonica	Tolerant
Sakha106	Giza 177/Hexi 30	Japonica	Sensitive
IRRI147	IRRI	Indica	Tolerant
IR65483-141-2-4-4-2-5	IRRI	Japonica	Tolerant
Egyptian Jasmine	IR262-43-8-11 x KDML 105	Indica	Moderate

**Table 2: Some chemical and physical analyses were estimated of experimental sites during 2022 season.**

pH (1:2.5 soil water suspension)	ECe (soil paste ) dSm <sup>-1</sup>	OM (organic matter%)	Soil texture	
8.35	8.39	1.43	Clayey	
Available micronutrients ppm (EDTA)	Fe	Zn	Mn	
	4.93	1.23	4.42	
<u>Soluble cations</u> meq l <sup>-1</sup> . (soil pates):	Ca <sup>++</sup>	Mg <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>
	7.43	5.32	2.36	62.09
<u>Soluble anions</u> meq l <sup>-1</sup> . (soil pates):	Co3 <sup>--</sup>	Hco3 <sup>-</sup>	Cl <sup>-</sup>	So4 <sup>--</sup>
	1.23	9.16	61.25	5.83

## Collected data

Data were recorded after complete heading. Twenty plants were taken randomly from the parents and F<sub>1</sub> crosses from each replicate to determine the morphological and yield and its components characters. At ripening stage, each plant was individually harvested. The following traits were measured for parent and their F<sub>1</sub> plants under salinity condition. The studied traits were; flowering date(days), plant height (cm), panicle length (cm), number of panicles plant<sup>-1</sup>, number of filled grains panicle<sup>-1</sup>, number of unfilled grains panicle<sup>-1</sup>, spikelet's sterility percentage panicle<sup>-1</sup>, panicle weight (gm.), 1000-grain weight (gm.), straw yield plant<sup>-1</sup> (gm.), harvest index (%) and grain yield plant (gm.).

## Statistical analysis

Line × tester analysis according to Kempthorne (1957) and explained by Singh and Chaudhary (1977) were used to estimate combining abilities under salt affected soil and heterosis over better parent.

## DNA extraction and amplification

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

## ISSR "Inter Sample Sequence Repeat"

### ISSR-PCR Reactions

Four ISSR primers were used in the detection of polymorphism. 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 dH<sub>2</sub>O, according to Zayed *et al.* (2023a). Furthermore, the sequence for the four ISSR markers was as follow;

ISSR-03(5'-ACACACACACACACACYT-3'),  
ISSR-10 (5'-GACAGACAGACAGACAAT-3'),  
ISSR-11 (5'-ACACACACACACACACYA-3') and  
ISSR-12 (5'-ACACACACACACACACYC-3')

## Thermocycling Profile PCR

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Bio-systems) 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. Jaccard 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 Jaccard similarity index using the PAST software Version 1.91 (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

The present study included the experiment concerned with the evaluation of seven parents and their twelve crosses under saline soil at the farm of El-Sirw Agricultural Research Station. This study investigated heterosis and combining ability for morphological, yield and its attributes as well as the genetic parameters of these characteristics.

### Analysis of variance

The results in Table 3 showed that insignificant effect was recorded among the replications for all studied traits. Highly significant variations were estimated among the tested rice genotypes and parents for all studied traits. The current results indicated that

genotypic differences and variation as well as large diversity among entries are very apparent and significant confirming its validation for further computation and hybridization. These results in agreement with those reported by El-Mowafi *et al.* (2022) and Zayed *et al.* (2023a).

**Table 3: Analysis of variance for line × tester design for all traits**

Source of variation	df	Flowering date (day)	Plant height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
Replications	2	0.75	2.12	0.88	0.54	4.27	5.43
Genotypes	18	109.87**	301.75**	12.08**	61.56**	311.99**	218.54**
Parents	6	128.38**	446.38**	15.07**	48.27**	296.69**	79.14**
Pa vs Cros	1	794.99**	1872.19**	43.91**	336.33**	2520.12**	2865.10**
Crosses	11	37.48**	80.09**	7.56**	43.83**	119.60**	53.98**
Lines	3	63.81**	104.19**	7.17**	124.99**	142.71**	93.89**
Testers	2	67.03**	110.25**	19.22**	10.33**	91.57**	102.92**
L x T	6	14.47**	57.99**	3.87**	14.41**	117.39**	17.71**
Error	36	0.64	5.62	0.38	1.08	5.34	3.28
Mean		100.51	103.12	23.48	23.56	93.05	26.60

**Table 3: Continue....**

Source of variation	df	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
Replications	2	4.41	0.99	0.002	22.56	2.37	3.00
Genotypes	18	87.43**	11.49**	0.366**	519.85**	31.57**	188.09**
Parents	6	73.79**	10.89**	0.144**	443.26**	31.30**	129.21**
Pa vs Cros	1	765.36**	76.91**	4.269**	4564.85**	10.28	1366.28**
Crosses	11	33.24**	5.88**	0.132**	193.90**	33.65**	113.09**
Lines	3	62.84**	4.31**	0.275**	181.42**	47.09**	281.50**
Testers	2	50.90**	11.00**	0.184**	579.58**	15.93*	73.94**
L x T	6	12.55**	4.95**	0.042**	71.58**	32.84**	41.94**
Error	36	2.46	0.51	0.005	10.13	4.18	4.43
Mean		21.83	23.84	2.242	65.84	32.60	32.09

Pa=parents, cros=crosses

However, mean squares due to line x tester was also highly significant for all the characters indicating that both additive and dominance or non-additive variances were important for those characteristics. These data are in harmony with those reported by Salgotra *et al.* (2009) and Vanave *et al.* (2018).

### Mean performance

The genotypes mean values for all traits under saline soil are presented in Table 4.

Concerning flowering date, the longest and undesirable mean values were obtained from the parent Egyptian Jasmine and the crosses Giza178×E.Jasmine and Giza178×IR65483. On the other side the best and earliest parent were GZ9399 followed by Giza179 and Sakha106 also, the results represented that the best crosses were GZ9399×IRRI147 and Giza179×IRRI147. Similar results were reported by Vanave *et al.* (2018), Negm *et al.* (2019), El-Mowafi *et al.* (2022) and Zayed *et al.* (2023a).

At the same time, the results in Table 3 showed that highly significant variation were obtained among crosses and parent *vs.* crosses for all studied traits, except harvest index differences between parent *vs.* crosses, which was insignificant. The current results could be used as indication to average heterosis overall crosses and through hybrid breeding technology to improve such traits (El-Mowafi *et al.*, 2022). Furthermore, the differences mean square values for lines and tester were significant and highly significant for all traits; these results proved the importance of additive and non-additive gene action effects. The highly significant variations between parents and crosses for all traits could be used as indication to average heterosis overall and also could be used through hybrid breeding technology to improve these traits. Kargbo *et al.* (2019) published similar results. Also, the mean squares due to lines and testers were highly significant for all studied characteristics indicating the predominance of additive variance.

**Table 4: The genotypes mean values for all traits under saline soil.**

Parents and crosses	Flowering Date (days)	Plant Height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
Giza178	98.00	95.33	21.50	23.33	98.00	16.33
Giza179	90.33	90.00	22.33	24.33	89.67	10.11
GZ9399-4-1-1-3-2-2	86.00	81.67	20.73	20.67	80.78	11.78
Sakha106	91.33	81.67	18.83	12.67	65.67	20.44
IRRI147	98.33	113.00	24.83	23.00	88.33	25.08
IR65483-14-1-4-13R	101.00	99.67	22.93	20.67	84.60	18.11
Egyptian Jasmine	104.33	108.00	25.17	18.00	83.33	19.33
Giza178×IRRI147	104.67	116.67	22.15	28.33	105.67	26.78
Giza178×IR65483	108.67	112.67	26.17	24.33	93.57	29.23
Giza178×E.Jasmine	108.33	101.67	23.18	28.33	100.57	37.78
Giza179×IRRI147	99.33	112.00	24.27	30.67	100.57	27.67
Giza179×IR65483	102.33	108.00	26.70	28.33	96.21	29.56
Giza179×E.Jasmine	104.67	110.67	23.67	26.33	106.42	29.78
GZ9399×IRRI147	96.00	107.67	23.50	28.00	101.23	27.44
GZ9399×IR65483	104.33	102.33	25.10	27.33	105.80	30.78
GZ9399×E.Jasmine	103.33	108.33	26.00	23.33	90.68	35.33
Sakha106×IRRI147	102.67	106.67	22.93	18.67	97.00	35.11
Sakha106×IR65483	105.00	105.00	24.40	21.00	87.11	37.22
Sakha106×E.Jasmine	101.00	98.33	21.77	20.33	92.67	37.44
LSD 0.05	0.76	2.26	0.59	0.99	2.20	1.72
LSD 0.01	1.02	3.02	0.79	1.33	2.95	2.31

Table 4: Continue...

Parents and crosses	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
Giza178	14.29	19.58	2.02	64.78	32.66	31.44
Giza179	10.06	23.00	2.15	62.07	35.22	33.63
GZ9399-4-1-1-3-2-2	12.68	22.37	1.84	43.89	35.40	24.00
Sakha106	23.58	24.63	1.47	36.11	27.99	13.89
IRRI147	22.13	21.01	1.85	69.33	29.44	28.91
IR65483-14-1-4-13R	17.63	24.60	2.04	47.22	34.76	25.33
Egyptian Jasmine	18.84	21.07	1.83	55.44	28.84	22.58
Giza178×IRRI147	20.22	23.44	2.35	74.67	37.39	44.33
Giza178×IR65483	23.81	25.67	2.69	82.54	31.96	38.78
Giza178×E.Jasmine	27.35	24.47	2.37	71.67	33.75	36.56
Giza179×IRRI147	21.57	23.43	2.64	86.00	33.71	43.78
Giza179×IR65483	23.50	26.03	2.85	75.20	34.54	39.67
Giza179×E.Jasmine	21.86	22.07	2.57	68.89	36.87	40.22
GZ9399×IRRI147	21.33	23.50	2.22	79.33	24.76	26.11
GZ9399×IR65483	22.53	25.77	2.46	68.33	33.79	34.89
GZ9399×E.Jasmine	28.04	26.33	2.39	61.89	31.32	28.22
Sakha106×IRRI147	26.58	26.03	2.41	77.22	30.70	34.22
Sakha106×IR65483	29.94	25.87	2.36	67.00	35.15	36.33
Sakha106×E.Jasmine	28.77	24.17	2.09	59.33	31.17	26.89
LSD 0.05	1.50	0.68	0.07	3.03	1.95	2.01
LSD 0.01	2.00	0.91	0.09	4.06	2.61	2.68

For plant height, the parent GZ9399 and Sakhsa 106 gave the lowest mean value (81.67 cm). On the other hand, the parent IRRI147 gave the highest value of plant height (113.0 cm). For crosses, the cross Sakha 106×E. Jasmine gave the lowest value of plant height (98.33 cm). Contrary, the crosses Giza178×IRRI147 and Giza178×IR65483 exhibited the highest mean values, since these values were 116.67 and 112.67cm, respectively. It is worthy to note that most of F<sub>1</sub> mean values were directed towards the tall parents. These in turn, suggested that tallness was dominant under saline soil condition (Negm, 2016).

With respect to panicle length, the most desirable mean values were obtained from the parents Egyptian Jasmine and IRRI147 beside the crosses, Giza179×IR65483 and Giza178×IR65483.

Concerning to number of panicles plant<sup>-1</sup>, the genotypes Giza179 gave the highest value of number of panicles per plant (24.33) followed by Giza178 (23.33). Among crosses, the results showed that Giza179×IRRI147 was confirmed its superiority under saline soil, since its value was (30.67) followed by Giza178×IRRI147, Giza178×E.Jasmine and Giza179×IR65483 with

value (28.33). Similar results were reported by Vanave *et al.* (2018), El Mowafi *et al.* (2022) and Zayed *et al.* (2023a)

With respect to filled grains panicle<sup>-1</sup>, the highest mean values were obtained from the genotype Giza178 (98.00) and Giza179 (89.67), as well as the crosses Giza179×E.Jasmine (106.42) and GZ9399×IR65483 (105.80). Similar results were reported by Vanave *et al.* (2018), El Mowafi *et al.* (2022) and Zayed *et al.* (2023a)

For number of unfilled grains panicle<sup>-1</sup>, the parent Giza179 recorded the lowest mean value of unfilled grains per panicle (10.11) followed by GZ9399 (11.78). Among the crosses, Giza178×IRRI147, GZ9399×IRRI147 and Giza179×IRRI147 gave the lowest values of unfilled grains panicle<sup>-1</sup> (26.78, 27.44 and 27.67), respectively.

For spikelet's sterility percentage, the lowest sterility percentage were recorded for the parent Giza179 followed by GZ9399, while the best crosses with lowest values for sterility percentage were Giza178×IRRI147 followed by GZ9399×IRRI147, Giza179×IRRI147 and Giza179×E.Jasmine without any significant differences between them.

With regarding to thousand grain weight, the highest mean value (24.63 g) was recorded by Sakha106 followed by IR65483 (24.60 g). For the crosses, GZ9399×E.Jasmine scored the highest value (26.33 g) followed by Sakha106×IRRI147 (26.03 g).

Concerning panicle weight, the most desirable mean value for this trait was obtained by the genotype Giza179 (2.15 g) followed by IR65483 (2.04g). It was noticed that the cross Giza179×IR65483 confirmed its superiority, since it gave the highest value of panicle weight (2.85 g) followed by Giza178×IR65483 (2.69 g).

Regarding straw yield plant<sup>-1</sup>, the rice variety IRRI147 occupied the first rank (69.33 g) followed by Giza178 (64.78 g). For the crosses, Giza179×IRRI147 confirmed its superiority, which gave the highest value of grain yield per

plant (86.00 g) followed by the cross of Giza178×IR65483 (82.54 g) comparing the rest crosses.

For harvest index, the most desirable genotypes recorded for Giza179, GZ9399, IR65483, Giza178×IRRI147 and Giza179× E. Jasmine, indicating the possibility to use these genotypes in breeding program to improve such trait under salinity condition.

As for grain yield plant<sup>-1</sup>, the parent Giza179 gave the highest mean value of grain yield per plant (33.63 g). The rice variety Giza178 occupied the second rank (31.44 g) followed by IRRI147 (28.91 g) without significant differences between them comparing to other parents. For the crosses, Giza178×IRRI147 confirmed its superiority, which gave the highest value of grain yield per plant (44.33 g) followed by the crosses of and Giza179×IRRI147 (43.78g) and Giza179×E.Jasmine (40.22 g). The current results confirmed the superiority and capability of Giza179 and Giza178 to grow under salt stress healthy owing to its high salinity withstanding (Zayed *et al.*, 2023b)

## Combining ability

### General combining ability effects

The results in Table 5 reveal that both parents Giza178, IR65483 and Egyptian Jasmine showed highly significant positive estimates of GCA effects for flowering date trait. Those parents are undesirable for this trait because it will inherit the late heading to its crosses which did not match with breeding goal. Furthermore, the genotypes: GZ9399 and IRRI147 were appeared good combiners for this trait in the terms of early maturing variety.

Both parents Sakha106 and Egyptian Jasmine showed significant negative estimates of GCA effects for plant height trait. On contrary, GCA of Giza178, Giza179 and IRRI147 were highly significant positive indicating these genotypes push toward tallness in their crosses which was not convenient with the purpose of breeding that prefers short plant type.

**Table 5: Estimates of general combining ability (GCA) of parents for all traits in rice**

Parents	Flowering date (day)	Plant Height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
Giza178	3.86**	2.83**	-0.32	1.58**	1.81*	-0.75
Giza179	-1.25**	2.72**	0.72**	3.03**	2.94**	-3.01**
GZ9399	-2.14**	-1.39	0.71**	0.81*	1.11	-0.83
Sakha106	-0.47	-4.17**	-1.12**	-5.42**	-5.87**	4.58**
LSD. (0.05)=	0.54	1.60	0.42	0.70	1.56	1.22
LSD. (0.01)=	0.73	2.15	0.56	0.94	2.09	1.64
IRRI147	-2.69**	3.25**	-0.94**	1.00**	2.99**	-2.76**
IR65483	1.72**	-0.50	1.44**	-0.17	-2.45**	-0.31
E. Jasmine	0.97**	-2.75**	-0.50**	-0.83**	-0.54	3.07**
LSD. (0.05)=	0.47	1.39	0.36	0.61	1.35	1.06
LSD. (0.01)=	0.63	1.86	0.49	0.82	1.81	1.42

**Table 5: Continue...**

Parents	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
Giza178	-0.83	-0.206	0.020	3.62**	1.44*	4.06**
Giza179	-2.31**	-0.887**	0.237**	4.02**	2.11**	5.39**
GZ9399	-0.66	0.469	-0.094**	-2.82*	-2.97**	-6.09**
Sakha106	3.80**	0.624*	-0.163**	-4.82**	-0.59	-3.35**
LSD.(0.05)=	1.06	0.482	0.046	2.15	1.38	1.42
LSD.(0.01)=	1.42	0.647	0.062	2.89	1.85	1.91
IRRI147	-2.20**	-0.629**	-0.047*	6.63**	-1.29*	1.28*
IR65483	0.32	1.102**	0.140**	0.60	0.94	1.58*
E. Jasmine	1.88**	-0.473**	-0.094**	-7.23**	0.35	-2.86**
LSD.(0.05)=	0.92	0.418	0.040	1.86	1.20	1.23
LSD.(0.01)=	1.23	0.560	0.054	2.50	1.61	1.65

For panicle length, the results also showed that highly significant positive estimate of GCA effects was obtained for parents Giza179, GZ9399 and IR65483. So, it could be expected that this parent contains the desirable genes for this trait. Zayed *et al.* (2023a) confirmed this result.

With regarded to Panicle number plant<sup>-1</sup>, significant and highly significant positive estimates of GCA effects were obtained for parents Giza178, Giza179, GZ9399 and IRRI147 (Table 5). These parents could be considered as good general combiners for this trait.



Concerning filled grains panicle<sup>-1</sup> the parents, Giza178, Giza179 and IRR147 which exhibited significant and highly significant estimates of GCA could be considered as good donors for improving this trait. The most important criteria contributing in the higher rice yield under salt stress is number of filled grains panicle<sup>-1</sup>, so it is very vital to use such crosses to improve the number of filled grains for rice growing under salt stress which will exert the lesser number of unfilled grains as seen in the next paragraph

Both parents Giza179 and IRR147 showed significant and highly significant negative values of GCA effects for unfilled grains panicle<sup>-1</sup> and spikelet's sterility percentage. So, these parents could be considered as a good general combiner for this trait under saline soils. On contrast, the parents Sakha106 and E.Jasmine showed highly significant positive values of GCA effect under salinity condition for these traits.

Regarding thousand grain weight, the best genotypes for combining ability was observed in IR65483 and Sakha106 indicating could use this parent in breeding program for improve the thousand grain weight under salinity condition

For panicle weight, two parents Giza179 and IR65483 were the good combiners since exhibit highly significant positive GCA effects and could use in breeding program to improve this trait.

For harvest index, Giza178 and Giza179 were the good combiners since exhibit significant and highly significant positive GCA effects and could use in breeding program to improve this trait.

Concerning straw and grain yield plant<sup>-1</sup>, the results showed that, the parents Giza178, Giza179 and IRR147 recorded significant and highly significant positive values of GCA effects. These parents could be strongly recommended as good general combiners for this trait especially in breeding programs for saline soils. The abovementioned combiners particularly, Giza178 and Giza179 are well known as salt tolerant varieties under Egyptian

condition in which the current results came also to confirm the previous fact in addition to their capacity to inherent their higher yield under such condition. Vanave *et al.* (2018), Negm *et al.* (2019), El Mowafi *et al.* (2022) and Zayed *et al.* (2023a) revealed similar result.

### Specific combining ability effects

The crosses of Giza179×IR65483, GZ9399×IRR147 and Sakha106×E.Jasmine showed highly negative significant specific combining ability estimates for flowering date (Table 6). From these results, it could be concluded that this cross could be utilized in rice breeding programs due to their desirable short duration.

The crosses GZ9399×IR65483 and Giza178×E. Jasmine exhibited significant and highly significant negative estimates of SCA for plant height. So that, this cross was could be utilized in rice breeding program owing to their desirable short stature.

With respect to panicle length, significant positive estimate of specific combining ability effects was found in the crosses Giza178×IR65483, Sakha106×IRR147 and GZ9399×E.Jasmine indicating its validation to improve these traits.

For number of panicle plant<sup>-1</sup>, the crosses GZ9399×IR65483, Giza178×E.Jasmine and Giza179×IRR147 have desirable significant positive SCA effects. The present nominated cross appeared to do well under such salt stress indicating its capability as source for plant material to keep high population and panicle number under salt stress to ensure high rice yield under such condition.

Regarding number of filled grains panicle<sup>-1</sup>, three crosses Giza178×IRR147, Giza179×E. Jasmine and GZ9399×IR65483 showed significant and highly significant positive specific combining ability estimates. These crosses and their parents could be used in breeding programs to improve this trait under saline condition.

**Table 6: Specific combining ability effects (SCA) of evaluated crosses under saline soil conditions for studied traits**

Crosses	Flowering date (day)	Plant Height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
Giza178×IRRI147	0.14	3.08*	-0.74*	0.33	2.74*	-1.73
Giza178×IR65483	-0.28	2.83*	0.89*	-2.50**	-3.91**	-1.72
Giza178×E.Jasmine	0.14	-5.92**	-0.15	2.17**	1.17	3.44**
Giza179×IRRI147	-0.08	-1.47	0.33	1.22*	-3.49*	1.43
Giza179×IR65483	-1.50**	-1.72	0.38	0.06	-2.40	0.87
Giza179×E.Jasmine	1.58**	3.19*	-0.71	-1.28*	5.90**	-2.30*
GZ9399×IRRI147	-2.53**	-1.69	-0.43	0.78	-1.00	-0.98
GZ9399×IR65483	1.39**	-3.28*	-1.21**	1.28*	9.02**	-0.09
GZ9399×E.Jasmine	1.14*	4.97**	1.63**	-2.06**	-8.02**	1.08
Sakha106×IRRI147	2.47**	0.08	0.84*	-2.33**	1.75	1.28
Sakha106×IR65483	0.39	2.17	-0.07	1.17	-2.70	0.94
Sakha106×E.Jasmine	-2.86**	-2.25	-0.77*	1.17	0.95	-2.22*
LSD. (0.05)=	0.94	2.78	0.73	1.22	2.71	2.12
LSD. (0.01)=	1.26	3.72	0.97	1.63	3.63	2.84

**Table 6: Continue....**

Crosses	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
Giza178×IRRI147	-1.37	-0.453	-0.076	-8.26**	4.31**	3.17*
Giza178×IR65483	-0.30	0.039	0.080*	5.65**	-3.34**	-2.69*
Giza178×E.Jasmine	1.68	0.414	-0.005	2.60	-0.97	-0.47
Giza179×IRRI147	1.46	0.218	-0.001	2.67	-0.04	1.28
Giza179×IR65483	0.87	1.087*	0.025	-2.09	-1.44	-3.14*
Giza179×E.Jasmine	-2.33**	-1.305**	-0.025	-0.58	1.48	1.86
GZ9399×IRRI147	-0.44	-1.071*	-0.094*	2.85	-3.91**	-4.91**
GZ9399×IR65483	-1.76	-0.535	-0.036	-2.11	2.90*	3.56**
GZ9399×E.Jasmine	2.19*	1.606**	0.131**	-0.73	1.01	1.34
Sakha106×IRRI147	0.35	1.307**	0.171**	2.74	-0.35	0.46
Sakha106×IR65483	1.19	-0.591	-0.069	-1.45	1.88	2.27
Sakha106×E.Jasmine	-1.54	-0.716	-0.101*	-1.29	-1.52	-2.73*
LSD. (0.05)=	1.84	0.835	0.080	3.73	2.39	2.46
LSD. (0.01)=	2.46	1.120	0.107	5.00	3.21	3.30

For unfilled grains panicle<sup>-1</sup> the cross of Giza179×E.Jasmine and Sakha106×E.Jasmine showed negative significant specific combining ability estimates. The current finding proved the validity of abovementioned crosses to overcome the problem of sterility induced by salinity stress, which affected the rice grain yield formation.

With respect to spikelet's sterility percentage, the cross of Giza179×E.Jasmine showed highly significant negative specific combining ability estimates considered as the best cross in this trait.

With respect to thousand grain weight and panicle weight, significant and highly significant positive estimate of specific combining ability effects were found in the crosses GZ9399×E.Jasmine and Sakha106×IRRI147 indicating their superiority as the best crosses for these traits under salt stress. Meanwhile, the crosses of Giza179×IR65483 and Giza178×IR65483 appeared significant positive SCA for thousand grain weight and panicle weight, respectively, which could be used in the breeding program for improving panicle characteristics under salt stress and subsequently rice grain yield.

With respect to straw yield plant<sup>-1</sup>, it could be noticed that highly positive significant specific combining ability estimates was appeared in the cross of Giza178×IR65483 indicating it as the best cross for this traits under such condition.

With respect to harvest index and grain yield plant<sup>-1</sup>, two crosses of Giza178×IRRI147 and GZ9399×IR65483 showed highly significant and significant positive regarding to SCA effects, indicating the possibility to successfully use them in breeding program under salinity condition for improving rice yield and enhancing their salinity withstanding.

### **Heterosis over better parents**

The heterosis percentage estimates over better parent for studied characters are listed in Table (7). For flowering date, plant height, unfilled grains panicle<sup>-1</sup> and sterility percentage unfortunately, the studied crosses were exhibited

highly significant positive heterosis indicating the hybrid vigor among these genotypes led to late maturity and tallness plants under salinity condition and also the genetic differences among them led to more unfilled grains and sterility. Similar results were confirmed by Negm *et al.* (2023).

Concerning panicle length, five of the twelve hybrids (Giza178×E.Jasmine, Giza179×IR65483, GZ9399×IR65483, GZ9399×E.Jasmine and Sakha106×IR65483) exhibited highly positive significant values for this trait. These five crosses could be used in breeding program to obtain the panicle length as a yield attributes which reflect on grain yield raising and also these results represent the importance of the parent IR65483 effect on improve the length of panicle in it crosses, the current findings were in completely agreement with those reported by Negm *et al.* (2023) and Zayed *et al.* (2023).

Regarding number of panicle plant<sup>-1</sup> and filled grains panicle<sup>-1</sup> the highly positive significant heterosis estimates were obtained by eleven hybrids out of the twelve crosses. This results indicating indicated an apparent improvement for panicle number and increase the filled grains in these crosses under saline soils as a result of new crosses.

Regarding to thousand grain weight, nine crosses out of twelve hybrid combination scored highly positive significant heterosis estimates over better parent indicating improve these traits and also the heterosis in the crosses will increase the grain yield.

For harvest index, the only five hybrids Giza178×IRRI147, Giza178×E. Jasmine, Giza179× E. Jasmine, Sakha106×IRRI147 and Sakha106×E.Jasmine scored highly positive significant heterosis estimates over better parent.

With respect to panicle weight, straw yield plant<sup>-1</sup> and grain yield plant<sup>-1</sup>, all crosses exhibited highly significant positive heterosis indicating high efficiency for improving panicle weight and grain yield in all studied crosses combination. This result prediction using these crosses in breeding program will be more effective.

**Table 7: Percentage of heterosis over better parents for studied characters studied under saline soil.**

Crosses	Flowering date (day)	Plant height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
Giza178×IRRI147	6.80**	22.38**	-10.81**	21.43**	7.82**	63.95**
Giza178×IR65483	10.88**	18.18**	14.10**	4.29**	-4.52**	78.99**
Giza178×E.Jasmine	10.54**	6.64**	-7.88**	21.43**	2.62*	131.30**
Giza179×IRRI147	9.96**	24.44**	-2.28**	26.03**	12.16**	173.63**
Giza179×IR65483	13.28**	20.00**	16.42**	16.44**	7.30**	192.28**
Giza179×E.Jasmine	15.87**	22.96**	-5.96**	8.22**	18.69**	194.46**
GZ9399×IRRI147	11.63**	31.84**	-5.37**	21.74**	14.60**	133.01**
GZ9399×IR65483	21.32**	25.31**	9.45**	32.26**	25.06**	161.31**
GZ9399×E.Jasmine	20.16**	32.65**	25.40**	12.90**	12.26**	200.00**
Sakha106×IRRI147	12.41**	30.61**	-7.65**	-18.84**	9.81**	71.75**
Sakha106×IR65483	14.96**	28.57**	6.40**	1.61**	2.97**	105.53**
Sakha106×E.Jasmine	10.58**	20.41**	-13.51**	12.96**	11.20**	93.68**

**Table 7: Continue...**

Crosses	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
Giza178×IRRI147	41.54**	11.56**	16.45**	7.69**	14.48**	40.99**
Giza178×IR65483	66.65**	4.34**	32.19**	27.42**	-8.04**	23.32**
Giza178×E.Jasmine	91.46**	16.14**	17.66**	10.63**	3.34**	16.25**
Giza179×IRRI147	114.41**	1.88	23.00**	24.04**	-4.30**	30.19**
Giza179×IR65483	133.59**	5.83**	32.95**	21.16**	-1.94	17.96**
Giza179×E.Jasmine	117.30**	-4.06**	19.72**	10.99**	4.67**	19.61**
GZ9399×IRRI147	68.25**	5.07**	20.01**	14.42**	-30.08**	-9.70**
GZ9399×IR65483	77.72**	4.74**	20.85**	44.71**	-4.55**	37.72**
GZ9399×E.Jasmine	121.20**	17.73**	29.81**	41.01**	-11.53**	17.60**
Sakha106×IRRI147	20.12**	5.68**	30.65**	11.38**	4.28**	18.36**
Sakha106×IR65483	69.85**	5.01**	15.89**	41.88**	1.14	43.42**
Sakha106×E.Jasmine	52.70**	-1.89**	14.61**	7.01**	8.07**	19.09**

### Estimation of genetic parameters and heritability

The estimates of genetic parameters, additive genetic variance ( $\sigma^2 A$ ), dominance genetic

variance ( $\sigma^2 D$ ), broad sense heritability ( $h^2b$  %), narrow sense heritability ( $h^2n$  %) are presented in Table 8. The results of additive variance ( $\sigma^2 A$ ) indicated that, these traits were largely governed

by additive gene action, while dominance variance ( $\sigma^2D$ ) are defined as including the non-additive genetic portion of total genetic variation arising largely from dominance and epistatic deviation. Pradhan *et al.* (2006) reported that general combining ability (GCA) is attributed to additive gene effects and additive x additive

epitasis and is theoretically fixable. On the other hand, specific combining ability is attributable to non-additive gene action that may be due to dominance, epitasis, or both and is non-fixable. The obtained results has similar trend with those indicated by Negm *et al.* (2023) and Zayed *et al.* (2023).

**Table 8: Genetic parameters were estimated of all traits and contribution of line, testers and line×testers.**

Parameters	Flowering date (day)	Plant height (cm)	Panicle length (cm)	Panicle number plant <sup>-1</sup>	Filled grain panicle <sup>-1</sup>	Unfilled grain panicle <sup>-1</sup>
$\sigma^2 A=$	1.99	1.91	0.32	2.54	0.19	3.13
$\sigma^2 D=$	4.61	17.46	1.16	4.44	37.35	4.81
$\sigma^2G$	6.59	19.36	1.48	6.98	37.54	7.94
$\sigma^2E$	0.64	5.62	0.38	1.08	5.34	3.28
$\sigma^2P$	7.24	24.99	1.86	8.06	42.88	11.22
cont. line	46.43	35.48	25.87	77.78	32.54	47.44
cont. tester	32.51	25.03	46.22	4.29	13.92	34.66
cont. l*t	21.06	39.49	27.91	17.93	53.54	17.90
*H. b=	91.11	77.50	79.41	86.59	87.55	70.79
*H. n=	27.43	7.63	17.09	31.49	0.44	27.89

**Table 8: continue...**

Parameters	Spikelet's sterility percentage	Thousand grain weight (g)	Panicle weight (g)	Straw yield plant <sup>-1</sup> (g)	Harvest index %	Grain yield plant <sup>-1</sup> (g)
$\sigma^2 A=$	1.78	0.08	0.008	10.55	0.07	6.14
$\sigma^2 D=$	3.36	1.48	0.013	20.48	9.55	12.50
$\sigma^2G$	5.15	1.56	0.020	31.03	9.62	18.64
$\sigma^2E$	2.46	0.51	0.005	10.13	4.18	4.43
$\sigma^2P$	7.61	2.07	0.025	41.17	13.80	23.07
cont. line	51.56	20.03	57.02	25.52	38.16	67.88
cont. tester	27.84	34.05	25.40	54.35	8.61	11.89
cont. l*t	20.59	45.92	17.58	20.14	53.23	20.23
*H. b=	67.63	75.39	81.25	75.39	69.72	80.80
*H. n=	23.45	3.87	30.83	25.64	0.51	26.61

The dominance component played a great role for all studied traits, where the dominance gene action ( $\sigma^2 D$ ) values were higher than additive variance. For environmental variance ( $\sigma^2 E$ ), the recorded estimates ranged from 0.005 for panicle weight to 10.13 for straw yield plant<sup>-1</sup>. In general, normal value of environment component was estimated for all studied characters but differed in its magnitude indicating that these characters are affected by the environment component with different degrees. The early selection in generation breeding is not recommended under saline conditions but it must be applied in late generations under controlled conditions to minimize environmental effects. In addition, the results in Table 8 showed also that the estimation recorded for genotypic variance ( $\sigma^2 G$ ), was 37.54 for filled grain panicle<sup>-1</sup>, while the lowest value was 0.020 which recorded by panicle weight. In addition to these results, the phenotypic variance ( $\sigma^2 P$ ) exhibited higher variance than the genotypic variance ( $\sigma^2 G$ ) in all yield and its components traits indicating the influence of environment in these traits beside the capability of genetic control of these traits.

Heritability in broad sense was high for most traits except harvest index and sterility percentage, indicating that most of phenotypic variability in all characters was due to genetic variation. The results indicated that the flowering date the highest trait didn't effect by environment and the genetic controlled on it since, heritability in broad sense more than ninety percent. Also, it could notice that the heritability estimates in narrow sense were relatively high for flowering date, panicle number plant<sup>-1</sup>, unfilled grain panicle<sup>-1</sup>, sterility percentage, panicle weight, straw yield plant<sup>-1</sup> and grain yield plant<sup>-1</sup>, indicating the importance of additive genetic variance. Moreover, the heritability estimates in narrow sense were relatively moderate for panicle length, these results also indicating the importance of both additive and dominance genetic variance. Also, the differences in magnitudes of broad and narrow sense heritability were indicating the presence of both additive and non-additive genetic variance in the most traits under study.

Agreement with the results that displayed high estimates of heritability and genetic variance were detected by Reddy (1992). Furthermore, Hammoud (2004) found that narrow sense heritability estimates were lower than broad sense for most characters studied indicating the importance of non-additive genetic variance in the inheritance of these characters; subsequently selection procedures are preferred in the late generation. The results also revealed that heritability in narrow sense were low for the thousand grain weight, harvest index, plant height, filled grain panicle<sup>-1</sup> traits, indicating that the dominance components played a great role for these traits; these results suggested that the selection must be done in the late generation.

The proportional contribution of lines, testers and their interaction to the total variance are presented in Table 8. It is evident that high contribution of lines is for the traits, flowering date, panicle number plant<sup>-1</sup>, unfilled grain panicle<sup>-1</sup>, sterility percentage, panicle weight and grain yield. Furthermore, the testers showed high contribution for panicle length, and straw yield. The contribution of crosses (line x tester) was found vital for plant height, filled grain panicle<sup>-1</sup>, thousand grain weight and harvest index. These results were conformity with Akhter *et al.* (2010) and Yuni *et al.* (2017). The significance of GCA (variances due to lines and testers) and SCA (variances due to lines vs. testers) implied that both additive and non-additive types of variation were available for all the traits.

### **Molecular marker (ISSR-PCR) and Cluster analysis based on ISSR marker**

Seven genotypes were amplified by PCR using four primers to determine the level of polymorphism in the seven rice parents that were evaluated (Fig. 1). The similarity matrix revealed that the two genotypes of japonica type rice, IR65483 and Sakha106, shared the maximum similarity percentage of 0.76, while GZ9399 and IR65483 shared the lowest similarity percentage of 0.43 (Table 9). Based on the analysis of ISSR data, a dendrogram of the genetic links between rice genotypes is shown (Fig. 2).

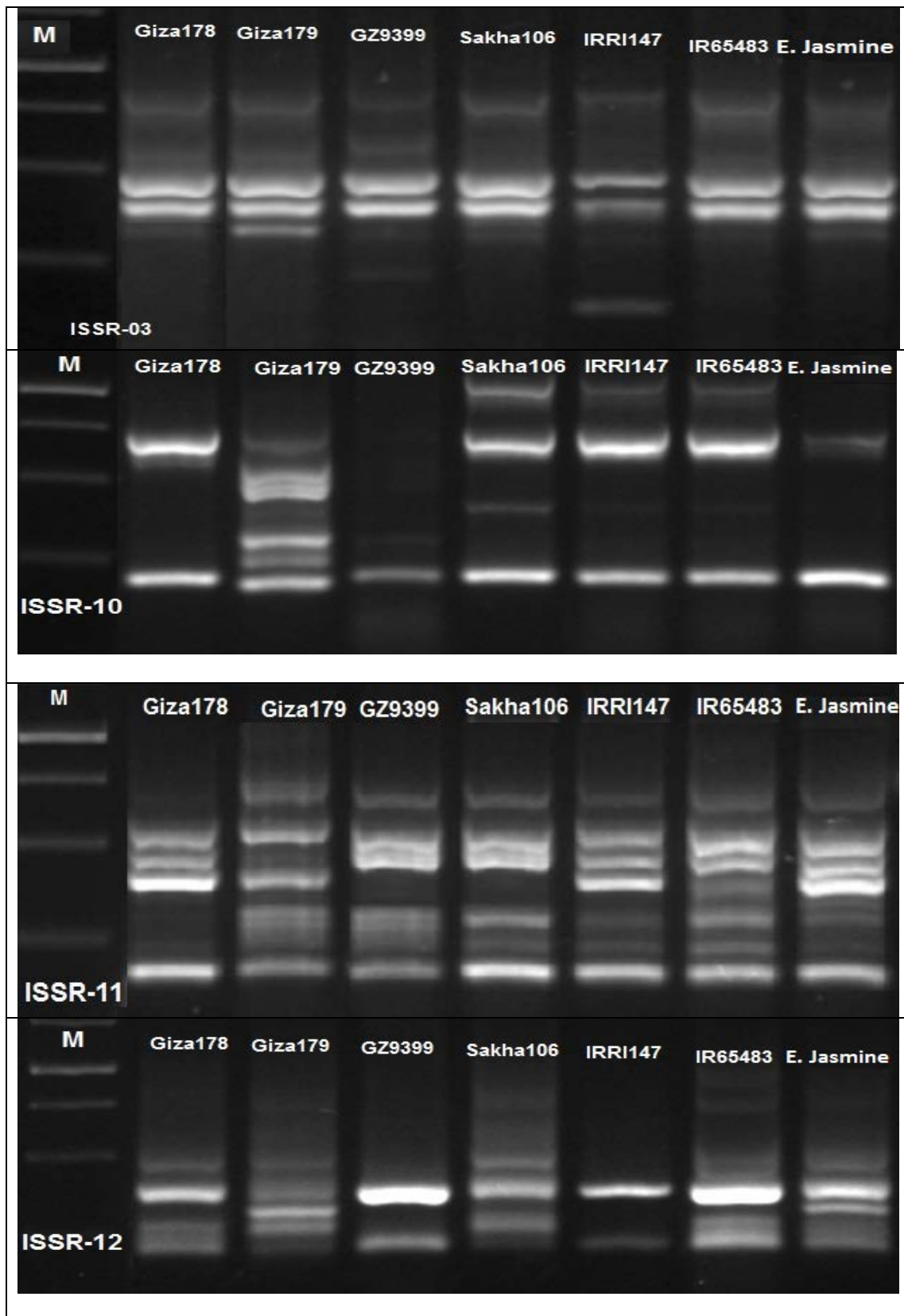
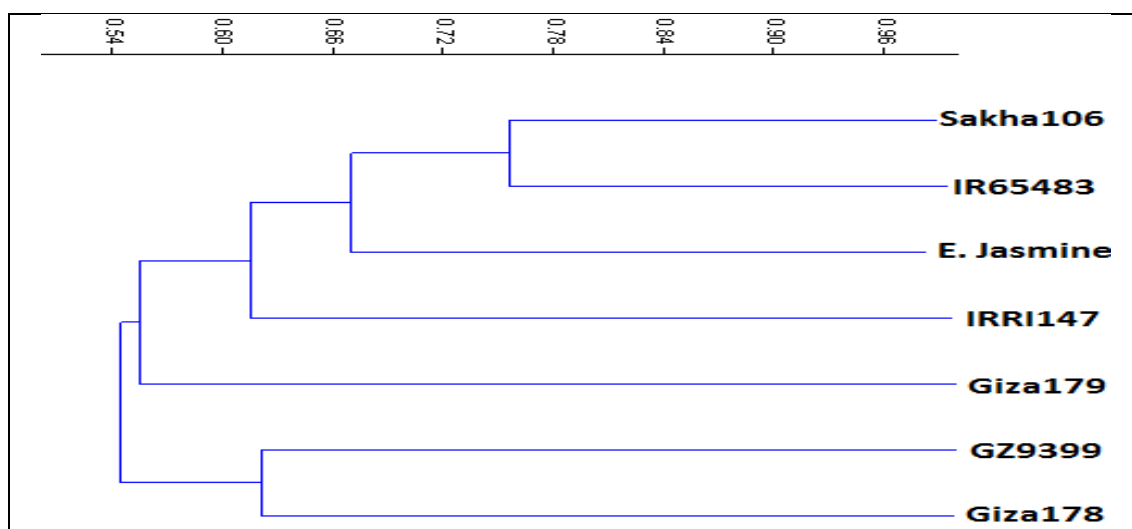


Fig. 1: ISSR profiles, the PCR patterns of the seven rice genotypes using the four ISSR Primers.

**Table 9: Similarity matrix among seven rice genotypes according to Jaccard coefficient as revealed by ISSR markers.**

Genotypes	Giza178	Giza179	GZ9399	Sakha106	IRRI147	IR65483
Giza179	0.60					
GZ9399	0.62	0.45				
Sakha106	0.61	0.60	0.54			
IRRI147	0.54	0.45	0.58	0.62		
IR65483	0.57	0.62	0.43	0.76	0.60	
E. Jasmine	0.58	0.55	0.55	0.66	0.63	0.68

**Fig. 2: Dendrogram for the seven rice genotypes constructed from ISSR data using UPGMA and similarity matrix computed according to Jaccard coefficient.**

The result in dendrogram revealed two major clusters; the first major cluster included the salt-tolerant indica/japonica rice varieties Giza178 and GZ9399. The second main cluster was divided into two sub-cluster clusters, one of which contained a single rice variety (Giza179); the other contained two groups, the first of which contained IRRI147, and the latter of which contained the remaining genotypes, with Egyptian Jasmine listed in a separate branch and the second branch containing Sakha106 and IR65483 with a similarity of the same order. These findings suggested that rice genotypes based on genetic resources and those that are tolerant to salinity can be identified and distinguished using ISSR markers. These findings concur with those of Zayed *et al.* (2023a).

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## دراسات وراثية وجزيئية على بعض الصفات المرتبطة بتحمل الملوحة في الأرز

محروس السيد نجم، عادل عطيه حديفه، سعيد عبد الغنى سلطان، شريف ماهر بسيونى

قسم بحوث الأرز ، معهد بحوث المحاصيل الحقلية ، مركز البحوث الزراعية، مصر.

### الملخص العربى

تم إجراء هذا البحث بمحطة البحوث الزراعية بسخا بكفر الشيخ وتربة طينية مالحة بالمزرعة التجريبية بمحطة بحوث السرو الزراعية مركز البحوث الزراعية بمحافظة دمياط في شمال الدلتا خلال موسمي ٢٠٢١ و ٢٠٢٢م. تم استخدام أربعة طرز وراثية من الأرز المصري هي (جيزه ١٧٨ وجيزه ١٧٩ و جى زد ٩٣٩٩) كتركيب وراثية متحملة للملوحة وسخا ١٠٦ حساس للملوحة كأم، واستخدمت ثلاثة تراكيب وراثية للأرز كآب (ايرى ١٤٧ و اى ار ٦٥٤٨٣) تم استخدام صنفين متحملين للملوحة والياسمين المصري (متوسط التحمل للملوحة). تم تهجين التراكيب الوراثية باستخدام تحليل السلالة في الكشاف وتم الحصول على اثني عشر هجيناً. تمت زراعة الجيل الاول وأبائهم وتقييمهم تحت ظروف الاراضى الملحية. كانت الصفات المدروسة هي ميعاد التزهير (أيام)، طول النبات (سم) ، طول السنابل (سم) ، عدد السنابل / نبات، عدد الحبوب الممثلة / السنبله، عددا لحبوب غير الممتلئه/ السنبله، نسبة عقم السنبيلات / السنبله ، وزن السنبله، وزن ١٠٠٠ حبة، محصول القش / نبات، ومحصول الحبوب / نبات دليل الحصاد. أشارت النتائج المتحصل عليها إلى أن الأباء جيزه ١٧٨ وجيزه ١٧٩ وايرى ١٤٧ كانوا أفضل الأباء في القدرة العامه على الانتلاف للصفات المدروسة تحت ظروف الاراضى الملحية. بينما كانت الهجن جيزه ١٧٩×ايرى ١٤٧ ، جيزه ١٧٨×ايرى ١٤٧ ، وجيزه ١٧٩×الياسمين المصرى الأفضل أداءً، وسيكون استخدامها تحت ظروف الاراضى الملحية أكثر فاعلية للحصول على سلالات واعدة. تم تقييم القرابه الوراثية بين سبعة أنواع مختلفة من الأرز باستخدام دلائل ISSR لتحديد الاختلافات الوراثية التي يمكن أن تؤدي الى قوه الهجين وانتخاب أفضل التراكيب الوراثية فى برامج التربية.