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IMPROVING SALT TOLERANCE RICE (*ORYZA SATIVA* L.) VARIETY BY CONVENTIONAL AND MOLECULAR BREEDING TO ENHANCE PRODUCTIVITY

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ABSTRACT: Salinity is a major constraint for sustainable rice production. Developing new rice varieties with inherent tolerance against these major abiotic stresses will help increase rice production under unfavorable conditions. The present study was conducted to create abiotic stress-tolerant rice genotypes in the genetic background of the popular rice variety improved Giza179 by pyramid hybridization and SSR markers. The results showed that all the basic parents IR1515-31, BG94-2 AC1225, Hua Lien Yu 202, GZ1368-5-S-4, and GZ6296 contributed to producing the new variety Giza179. The analysis of variance showed highly significant differences for all the studied traits under saline soil conditions except root length and milling percentage. The main performance showed that the salinity conditions at the Lysimeter site negatively affected the yield trait for all rice genotypes compared with normal soil. The phenotypic coefficient of variability (PCV%) was greater than the genotypic coefficient of variability (GCV%) in all genotypes for all traits over two years. This means that most of the PCV% was influenced by environmental factors and cultural practices. Twelve SSR markers were identified, and association analysis revealed that the markers RM1212, RM9, RM493, and RM23 had the highest PIC values.

Keywords: Rice, abiotic stress, pyramid breeding, molecular markers.

INTRODUCTION

Rice (Oryza sativa L.) is a principal food source globally, with over half of the world's population reliant on it (Sharma *et al.* 2020). On the other hand, the world population is rapidly increasing with each passing year, and increasing rice production will be needed to meet the population increase and reduce the gap between consumption demand and annual production (Kromdijk and Long, 2016). However, there are many constraints to increasing rice production, such as shortage of water, salinity, climatic changes, diseases, and insects (Almeida *et al.*, 2016 and Suvi *et al.*, 2021). Salinity has the greatest impact on production because it affects large areas of agricultural land, particularly those near seas and oceans, or lands that have been newly reclaimed (Kibria and Hoque 2019). Breeding for the improvement of new rice cultivars through the crossing method is considered the most economical way to produce new varieties and yield under saline soil conditions is an important indicator to evaluate the merits of rice varieties (Qin *et al.*, 2020).

In Egypt, the breeding program for salinity tolerance depends on evaluating the segregation generations, starting from F_2, F3, F_4, and F_5 generations under the conditions of salinity. It was produced through these two varieties; namely, Giza 178, which was produced from crosses 171 with Milyang 49, and Sakha104 produced from crosses GZ4096 with GZ4100, this method is considered the most effective way to create new cultivars and is called the conventional breeding (Sakina *et al.*, 2016; Qin *et al.*, 2020 and Chen *et al.*, 2021). However, this method requires knowledge of the nature of gene action governing the trait, and additive gene

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action is correlated to narrow sense heritability (Mohammadi *et al.,* 2014; Souleymane *et al.,* 2017). Thus, significant additive gene action means high heritability and implies selection at early generation on a plant basis. In the last few years, success in improving salt tolerance in rice relied on a remarkable effort to identify a major quantitative trait locus (QTL) contributing to salt tolerance in rice (Hoang *et al.,* 2016). However, a total of 2681 genes in these QTL intervals made it difficult to pinpoint the genes responsible for the functional differences in the traits (Turan *et al.,* 2012).

Finally, conventional breeding, and other innovative breeding techniques such as tissue culture, induced mutations, and wide crosses needed to be explored. The tools of biotechnology (specifically, molecular marker-aided selection (MAS) and genetic engineering) offer a promise to complement existing breeding strategies (Rana, *et al*., 2019; Sun, *et al*., 2019 and Yuan, *et al*., 2020). Plant breeding based on classical methods of hybridization and selected molecular marking methods are now widely used to allow the identification of the target genes and the selection of the desired genotypes (Kostylev *et al.* 2018). This investigation describes the procedures carried out to develop the new high-yielding variety Giza179 through pyramiding of favorable salt tolerance genes from multiple donors, identification of superior salt-tolerant alleles from multiple donors for rice improvement, evaluation of these lines for salt tolerance and agronomic performance in greenhouse and field experiments will be done to identify high yielding breeding lines with enhanced salt tolerance.

MATERIALS AND METHODS

Plant Materials and Crosses design

Four rice genotypes namely, GZ1368 and GZ6296 these two promising lines crossing and produced Giza179, while Giza178 was used as a check variety. All the genotypes were selected and collected from breeding materials at Rice Research and Training Center (RRTC), Sakha Research Station, Kafr El-Sheikh, Agricultural Research Center, Egypt (Table 1). However, the first one GZ1368-5-S-4 was produced for cross IR1515-31/BG94-2 and the second parent was GZ6296, which was produced from cross AC1225/Hua Lien Yu 202.

Table (1): Parentage, Origen, types, and reaction of salinity tolerant of four rice varieties.

No.	Genotypes	Parentage	Origen	Type	Types of tolerant	Salinity score*
1	Giza 179	GZ1368-5-S-4 / GZ6296	Egypt	Indica Japonica	Tolerant	3
2	GZ1368	IR1515-31 / BG94-2	Egypt	Indica Japonica	Tolerant	3
3	GZ6296	AC1225 / Hua Lien Yu 202	Egypt	Indica Japonica	Moderate	2
4	Giza 178(Check)	Giza 171 / Milyang 49	Egypt	Indica Japonica	Tolerant	3

*=According to IRRI scale2002

Experimental procedure

Parental genotypes of this investigation were sown in the summer season in three sowing dates, at 15-day intervals to overcome the difference in heading date among the parental varieties. After 30 days of sowing, the seedlings of the parents were transplanted to the experimental field in three rows, five meters long and 20 x 20 cm apart between plants and rows. The method of hybridization technique was used according to Butany (1961) and the hot water method of emasculation was utilized. The resulting crosses were evaluated and arranged in a randomized complete block design (RCBD) experiment with three replications.

Segregation population

The F1 to F5 generations were evaluated to derive new lines that exhibit high yield and salinity tolerance. Normal conditions were observed at the Sakha location, whereas saline conditions were present in the Lysimeter. The levels and concentration of required salinity are controlled by drainage and washing the soil used. The salinity was adjusted to 3000 ppm, and the control was irrigated with tap water. Irrigation water, at 25°C, had an electrical conductivity (EC) of 0.77 dS/m for tap water and 10.6 dS/m for salinity. The water was artificially salinized by applying sodium chloride (NaCl) and calcium chloride ($CaCl_2$) in a 2:1 ratio, respectively (Soltan 2006 and Hassan 2013). The Lysimeter is concrete beds (1m width x 2m length) filled with soil to 100 cm depth in three layers: 60 cm clay at the surface, 20 cm sand at the middle, and 20 cm gravel at the bottom. The soil was sampled before cultivation, and a part of it was stored in the refrigerator for chemical analysis and the other for physical analysis according to Cottein *et al.* (1982) and Page *et al.* (1982). The physical and chemical properties of the experimental field soils are given in Table 2.

Table (2): Physical and chemical analysis of the soil at experimental sites during 2023 and 2024.

Properties / Locations	pH	EC (dS/m)	Soil texture	Cautions				Anions		
				Na^+	Ca^{++}	Mg^{++}	K^+	HCO_3^-	Cl^-	SO_4^{--}
(Normal soil)	8.00	2.42	clayey	11.1	5.2	4.8	0.5	6.0	10.6	7.5
(Saline soil)	8.16	9.41	clayey	61.4	15.2	16.3	1.2	7.0	53.0	34.1

Quantitative Traits Assessment

Thirteen agronomic traits namely; duration (day), plant height (cm), chlorophyll content, root length, root volume, number of panicles per plant, panicle length (cm), 1000-grain weight (g), sterility percentage, grain yield (t/ha), hulling, milling, head rice, and amylose percentage were calculated under normal and saline soil at the experimental farm. The data for all traits were recorded based on the Standard Evaluation System (SES) for rice (IRRI, 2002). The averages of two years of these characters were used for constructing genetic relationships among rice genotypes under the study of the parental genotypes.

Molecular Marker Analysis

Four rice genotypes were employed in the study to identify the salinity genes by using four specific primers namely, RM212, RM9, RM493, and RM23 purchased from Sangon Company, China. Molecular analysis was conducted at the EPCRS Centre (Certified according to ISO 9001, ISO 14001, and OHSAS 18001), Kafr El-Sheikh University, Egypt. DNA was isolated from the parents and selected lines from six populations according to Maixner *et al*. (1995). Selected symptomatic leaves were washed with clear water. 4 to 10 midribs were cut with a disposable razor blade and 1.0 g of midribs were dispensed in an ELISA sachet and 3.0 mL CTAB extraction buffer. Then the midribs were squashed under cooling at 4ºC. 1.5–2.0 mL of midrib juice was transferred to a 2 mL tube and kept in a water bath at 65ºC for 15 min. This was followed by centrifugation at 3000 in 5 min. One mL of supernatant was collected and transferred to an Eppendorf tube. 1 mL Chloroform-Isoamylic alcohol was then added and mixed by inverting the tubes several times to obtain an emulsion. The emulsion was then kept in the Centrifuge for 5 min at 14000 ×g. The aqueous phase was collected and transferred to new tubes, and 540 µL Isopropanol was supplied, left at - 20ºC for 30 min, and then centrifuged for 20 min at 14000 ×g. After centrifugation, ethanol was removed without disturbing the small nucleic acid pellet. The pellet was washed with 1 mL ethanol 70%, followed by centrifuge for 10 min at 14000 ×g. Ethanol was removed, and the pellet was dried at speed-VAC*5 min. The dried pellet was re-

suspended in 60-100 µL of TE 1X (Tris 10 mM, EDTA 1 mM, pH 8) and the nucleic acid was stored at -20ºC.

Polymerase Chain Reaction Assay

The reaction mixture (25 µL) consisted of: 12.5 µL of 2x master mix ready to use (0.1U/µL Taq Polymerase, 500 µM dNTP, 20 mM Tris-HCl (pH8.3), 100 mM KCl, 3 mM $MgCl_2$ and Stabilizer and enhancer) + 10 Pmol of each primer (1.0 µL) + 1.0 µL of DNA (50 ng) + 9.5 µL PCR grade water. Amplification was performed in a Thermocycler (Bio-Rad, C - 1000) as follows: (1). Initial denaturation at 94ºC for 5 min. (2) Denaturation at 94 ºC for 30 sec. (3) Primers annealing temperature differing according to Tm of each gene for 1 min. (4) Extension at 72 ºC for 1 min. (5) Steps 2, 3, and 4 are repeated 40 cycles. (6) A final extension at 72 ºC was given for 10 min. After PCR, the amplified products were analyzed on 1.5% agarose gel containing ethidium bromide to a final concentration of 0.5 µg mL-1 as follows: electrophoresis grade agarose (0.9 g) was prepared in 60 mL TAE 1x buffer in a sterile flask. It was heated in a microwave to dissolve all granules with agitation and allowed to cool at 70 ºC, and then 0.5 µg mL-1 ethidium bromide was added and mixed thoroughly. The warm agarose was poured directly into the gel casting apparatus with the desired comb in apposition and left at room temperature for polymerization. The comb was then removed, and the electrophoresis tank was filled with TAE (1x) buffer. Ten µL of amplified product was loaded into the well and ran along with 1 Kb plus DNA ladder (Intron Biotechnology Company, Korea) in a 1x TAE electrophoresis buffer at 5 volts/cm2 for 45 min. After the electrophoresis, the gels were transferred to a UV cabinet. The gels were then photographed and analyzed, using BioDoc Analysis software (Biometra, Germany).

Statistical analysis

The data averages were statistically analyzed using a two-way analysis of variance (ANOVA) with a complete randomized block design, according to Panse and Sukhatme (1985). Significant values were decided using p values ($p<0.05$ = significant and $p<0.001$ = highly significant). Also, correlation coefficients (r) among all studied traits were computed according to Gomez and Gomez (1983). Finally, Least Significant Difference (LSD) values were estimated according to the formula suggested by Wynne, *et al.,* (1970). The estimates of genetic parameters such as GV, PV, PCV%, and GCV% were computed based on the formula suggested by Burton (1952). According to the formula suggested by Hanson *et al* (1956), broad-sense heritability was calculated. Genetic advances were calculated and categorized by Johnson *et al.* (1955). The cluster analysis utilizing genetic distance was conducted with the PAST software package, employing the mean performance of rice genotypes (Hammer *et al.* 2001).

RESULTS AND DISCUSSION

Pyramiding genes from multiple donors to enhance salt tolerance is economical and the best way to obtain new durable tolerant verity, but the main obstacle to designing salt-tolerant cultivars is the narrow genetic basis for the use of rice germplasm. The result of this study showed that the traditional breeding methods were used to produce a new tolerant variety through pyramiding crosses (Fig.1). The first crosses to produce two promising lines namely, GZ1368-5-S-4 (Tolerant for salinity and produced from the cross between IR1515-31 and BG94-2) and GZ6296 (moderate for salinity and produced from the cross between AC1225 and Hua Lien Yu 202) these lines belonged to Indica Japonica types. The F_1 generation to the F_5 generations were evaluated under saline soil conditions to obtain salinity-tolerant lines. The second cross was between the two promising lines GZ1368-5-S-4 and GZ6296 to produce the Giza179 variety (Fig.1).

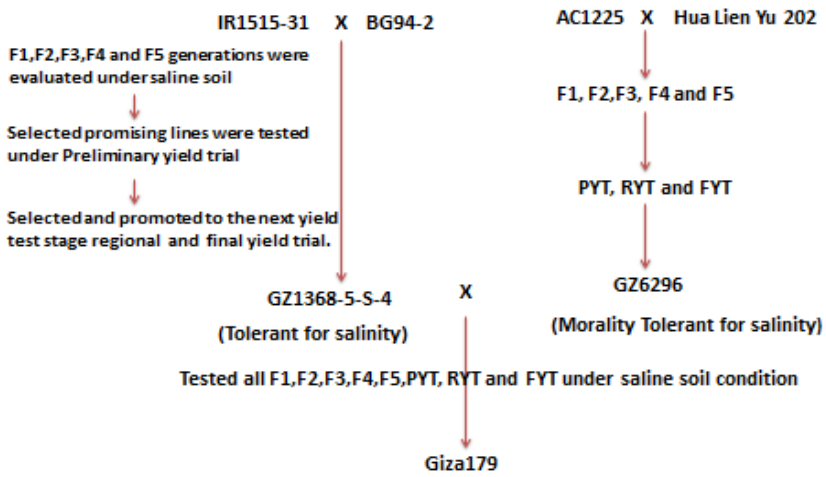

Figure 1. Pyramiding crosses for production Giza 179 variety (Tolerant for salinity and registered in 2017).

Analysis of variance

The analysis of variance (Table 3) exhibited highly significant differences for all the studied traits under saline soil conditions except root length and milling percentage, indicating a wide range of genetic variability among the studied genotypes, which is a primary requirement for further computation. While, under normal soil, all the traits were significant and highly significant differences except chlorophyll content, number of panicles per plant, and Sterility percentage.

Table 3. Analysis of variance for physio-morphological traits in four rice genotypes.

S.O.V	d.f	Duration (day)		Plant height (cm)		Chlorophyll content		Root length		Root volume	
		N	S	N	S	N	S	N	S	N	S
Replications	2	1.08^{ns}	0.58^{ns}	0.33^{ns}	11.76^{*}	0.48^{ns}	6.55^{**}	0.71^{ns}	0.44^{ns}	1.03^{ns}	0.15^{ns}
Genotypes	3	136.11^{**}	65.11^{**}	50.66^{**}	344.01^{**}	8.66^{ns}	15.42^{**}	11.86^{*}	4.52^{ns}	48.81^{**}	23.42^{**}
Error	6	0.52	0.69	1.66	1.05	7.53	0.09	1.48	1.14	0.84	2.38
Analysis of variance for yield and its component traits in four rice genotypes.											
S.O.V	**d.f**	**No. of panicles plant^{-1}**		**Panicle length (cm)**		**1000-grain weight (g)**		**Sterility %**		**Yield (t/ha)**	
		N	S	N	S	N	S	N	S	N	S
Replications	2	0.58^{ns}	2.33^{ns}	0.20^{ns}	1.01^{ns}	1.10^{ns}	0.06^{ns}	0.30^{ns}	0.54^{ns}	0.01^{ns}	0.12^{ns}
Genotypes	3	2.22^{ns}	9.33^{*}	1.90^{**}	2.22^{*}	21.47^{**}	15.42^{**}	1.47^{ns}	7.03^{*}	7.62^{**}	0.89^{**}
Error	6	1.14	1.66	0.45	0.39	0.03	0.06	0.38	1.45	0.07	0.03
Analysis of variance for grain quality traits in four rice genotypes.											
S.O.V	**d.f**	**Hulling %**		**Milling %**		**Head rice %**		**Amylose**			
		N	S	N	S	N	S	N	S		
Replications	2	0.50^{ns}	0.18^{ns}	0.07^{ns}	0.88^{ns}	0.14^{ns}	4.42^{*}	0.36^{*}	0.76^{ns}		
Genotypes	3	6.28^{*}	4.39^{*}	4.54^{**}	1.03^{ns}	3.06^{**}	3.04^{**}	12.85^{**}	7.52^{*}		
Error	6	0.21	0.62	0.20	0.44	0.30	0.04	0.05	0.16		

Mean performance

Data in Table 4 showed that the Giza179 variety was the earlier one and the duration was 122 days under normal saline soil, while GZ1368 was the long duration one under normal soil. For plant height, the salinity condition harms plant height for all rice genotypes under study compared with normal conditions (Sakha). Plant height increased significantly under normal soil which will improve rice growth, photosynthesis, metabolism, and assimilates production leading to improving plant height as a result of raising cell elongation (Metwally *et al*., 2010). Concerning chlorophyll content, the results

indicated that salinity soil affected significantly chlorophyll content and normal soil (Sakha) produced higher chlorophyll content values than Lysimeter (saline soil) location Chlorophyll content. These results cleared that chlorophyll content increased by fertile soil that is attributed to enhancing chlorophyll formation and it is favorable effect on assimilates production. The present results are similar to those claimed by Luo and Li (2000). The rice genotype GZ6296 gave the best value (44.8) of chlorophyll content compared with the others under the two conditions. As for root length, the two varieties Giza 178 and Giza 179 gave the highest value under normal and saline soil, while root volume the varieties G178 and GZ6296 gave the highest value under the two conditions. According to the number of panicles per plant, panicle length, and 1000-grain weight, Giza179 gave the highest value under normal and saline soil conditions, indicating that this genotype is suitable for growing under saline and normal conditions and it could be used to improve these traits in rice breeding program. In grain yield (t/ha) are presented in (Table 4). The results indicated that the salinity conditions at the Lysimeter site adversely impact the yield trait of all rice genotypes compared to normal soil. The Giza 179 variety demonstrated high yields in both normal and saline soils. On the other hand, Giza178 and GZ6296 gave the highest values of hulling, milling, and head rice percentage traits under normal and saline soil (Table 4). For amylose content, the genotype gave a high value under saline soil compared with normal soil; this indicated that salinity stress was affected by grain quality components.

Table (4): Mean performance for physio-morphological traits in four rice genotypes.

Genotypes	Duration (day)		Plant height (cm)		Chlorophyll content		Root length		Root volume	
	N	S	N	S	N	S	N	S	N	S
Giza 179	122.3	123.6	98.0	73.0	42.4	34.3	30.8	23.6	78.9	59.5
Giza 178	134.3	133.0	105.3	80.9	43.2	36.6	32.5	25.4	87.3	60.4
GZ1368	138.0	132.0	96.0	90.5	40.8	31.5	27.9	22.6	78.5	54.4
GZ6296	130.0	129.6	98.0	97.4	44.8	35.9	29.3	24.8	81.5	56.4
LSD 0.05	1.15	1.32	2.05	1.62	4.35	0.49	1.93	1.70	1.45	2.45
0.01	1.86	2.13	3.31	2.62	7.04	0.80	3.12	2.74	2.35	3.96
Mean performance for yield and its component traits in four rice genotypes.										
Genotypes	**No. of panicles plant^{-1}**		**Panicle length (cm)**		**1000-grain weight (g)**		**Sterility %**		**Yield (t/ha)**	
	N	**S**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
Giza 179	25.3	19.0	24.3	20.5	27.5	25.4	4.5	13.9	12.1	5.4
Giza 178	22.0	17.6	22.1	20.4	21.8	20.3	6.1	15.4	11.5	5.0
GZ1368	21.3	15.0	23.0	19.5	23.0	21.1	5.5	12.0	8.5	4.2
GZ6296	22.6	17.0	23.9	18.6	26.3	24.8	5.9	12.5	11.1	5.1
LSD 0.05	1.69	2.04	1.07	0.99	0.29	0.40	0.98	1.91	0.43	0.30
0.01	2.73	3.31	1.73	1.61	0.46	0.56	1.59	3.09	0.69	0.50
Mean performance for grain quality traits in four rice genotypes.										
Genotypes	**Hulling %**		**Milling %**		**Head rice %**		**Amylose**			
	N	**S**	**N**	**S**	**N**	**S**	**N**	**S**		
Giza 179	77.9	77.6	69.6	66.0	60.7	52.3	17.9	19.3		
Giza 178	80.5	78.9	71.1	66.9	62.8	55.4	21.7	22.8		
GZ1368	78.4	76.0	68.7	66.1	62.6	56.6	18.7	22.0		
GZ6296	80.7	77.0	71.2	67.2	62.7	57.8	17.9	20.5		
LSD at 0.05	0.73	1.24	0.71	1.05	0.87	0.30	0.34	0.63		
0.01	1.18	2.02	1.15	1.70	1.41	0.48	0.56	1.03		

Phenotypic, genotypic variability, heritability, and genetic advance

Estimates of phenotypic, genotypic coefficient variability, heritability, and genetic advance are presented in (Table 5). The results showed that most of the traits under study had a wide range of variability. This wide range was reflected in the variation among tested cultivars, where all cultivar's mean squares for all traits were highly significant under normal and saline

soil. Thus, selection for given traits among these cultivars would be effective in all cases (Hammoud 2009, Metwally *et al* 2010 and Hassan 2013). The phenotypic coefficient variability (PCV%) was greater than the genotypic coefficient variability (GCV%) in all genotypes for all traits over two years. This suggests that most of the PCV% was influenced by environmental conditions and cultural practices. The phenotypic coefficient of variability (PCV %) and genotypic coefficient of variability (GCV %) for plant height are presented in (Table 5), the results showed that the phenotypic coefficient of variability PCV% was higher than the genotypic coefficient of variability GCV% under both soils. However, the GCV% values ranged from 0.36 to 45.19% for normal soil and 0.19 to 21.47% for saline soil.

Table (5): Estimates of genetic components for physio-morphological traits in four rice genotypes.

Genetic parameters	Traits									
	Duration (day)		Plant height (cm)		Chlorophyll content		Root length		Root volume	
	N	S	N	S	N	S	N	S	N	S
Genotypic variance (GV)	45.19	21.47	16.33	14.32	0.37	5.11	3.46	1.12	15.99	7.01
Phenotypic variance (PV)	45.72	22.16	18.00	15.36	7.91	5.20	4.94	2.27	16.83	9.39
Genotypic coefficient of variation (GCV)	5.12	3.58	4.06	12.50	1.43	6.53	6.16	4.39	4.90	4.58
Phenotypic coefficient of variation (PCV)	5.15	3.64	4.27	12.56	6.56	6.59	7.36	6.23	5.03	5.31
Heritability (H_{bs} %)	98.8	96.8	90.7	99.0	4.7	98.1	70.0	49.5	95.0	74.6
Genetic advance (GA)	13.76	9.39	7.93	21.92	0.27	4.61	3.20	1.53	8.03	4.71
Genetic advance in percent of the mean (GA%)	10.49	7.26	7.98	25.64	0.64	13.32	10.62	6.36	9.84	8.16

Estimates of genetic components for yield and its component traits in four rice genotypes.

Genetic parameters	Traits									
	No. of panicles plant^{-1}		Panicle length (cm)		1000-grain weight (g)		Sterility %		Yield (t/ha)	
	N	S	N	S	N	S	N	S	N	S
Genotypic variance (GV)	0.36	2.55	0.48	0.61	7.14	5.12	0.36	1.85	2.51	0.28
Phenotypic variance (PV)	1.50	4.22	0.94	1.00	7.17	5.18	0.74	3.31	2.59	0.32
Genotypic coefficient of variation (GCV)	2.69	9.59	3.04	3.93	10.82	9.97	10.83	10.10	14.65	10.72
Phenotypic coefficient of Variation (PCV)	5.48	12.32	4.25	5.05	10.84	10.03	15.55	13.48	14.86	11.39
Heritability (H_{bs} %)	24.07	60.52	51.25	60.74	99.54	98.75	48.50	56.10	97.16	88.63
Genetic advance (GA)	0.61	2.56	1.02	1.25	5.49	4.63	0.86	2.10	3.22	1.03
Genetic advance in percent of the mean (GA%)	2.71	15.37	4.48	6.32	22.24	20.41	15.54	15.58	29.76	20.79

Estimates of genetic components for grain quality traits in four rice genotypes.

Genetic parameters	Traits							
	Hulling %		Milling %		Head rice %		Amylose	
	N	S	N	S	N	S	N	S
Genotypic variance (GV)	2.02	1.25	1.45	0.19	0.91	1.00	4.26	2.45
Phenotypic variance (PV)	2.23	1.87	1.65	0.63	1.22	1.04	4.31	2.61
Genotypic coefficient of variation (GCV)	1.80	1.44	1.72	0.66	1.54	1.76	10.96	7.39
Phenotypic coefficient of variation (PCV)	1.88	1.77	1.82	1.20	1.77	1.80	11.02	7.63
Heritability (H_{bs} %)	90.41	66.99	87.75	30.70	75.18	96.50	98.90	93.83
Genetic advance (GA)	2.78	1.89	2.32	0.50	1.71	2.02	4.23	3.12
Genetic advance in percent of the mean (GA%)	3.51	2.44	3.30	0.75	2.75	3.56	22.45	14.75

Correlation coefficient

The study of relationships among morphological root characters is of great importance. The estimated values, of the correlation coefficient among all studied characters, are presented in (Figure 2). Regarding correlations under normal soil, there are highly significant and positive correlations between plant height and each root length, root volume, and amylose content. Chlorophyll content showed a highly significant and positive correlation with milling percentage. On the other hand, the grain yield trait recorded a significant correlation with some other traits like root length and number of panicles/plants. Concerning the correlation coefficient under saline soil, root volume trait showed a highly significant and positive correlation with sterility % and hulling %. For grain yield, results showed a highly significant positive correlation coefficient between this trait and the number of panicles per plant under salinity. Also, significant correlations were recorded between grain yield and chlorophyll content, root volume, and 1000-grain weight.

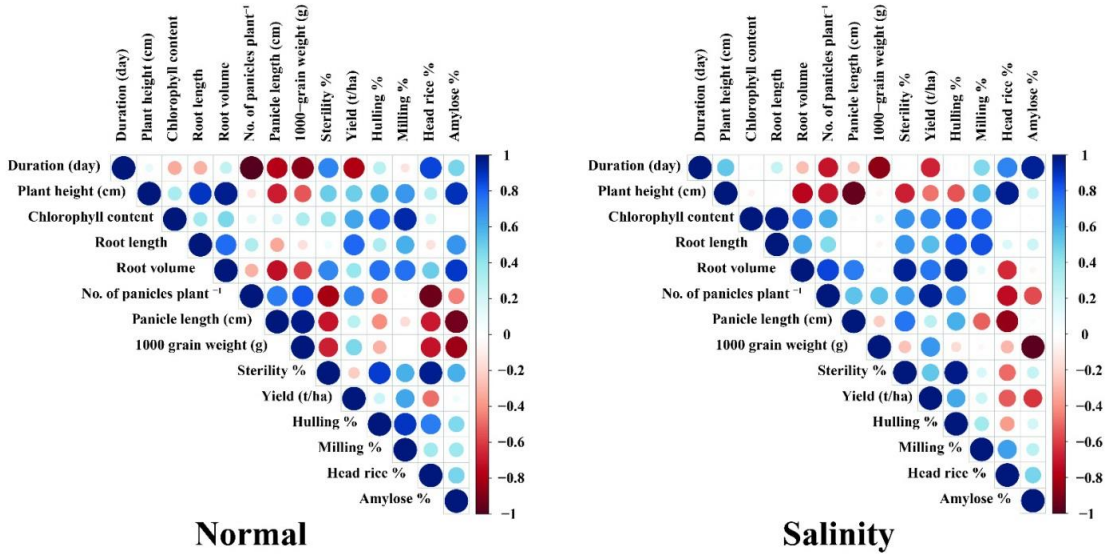

Figure 2. Estimates of phenotypic correlation coefficients among all studied characters.

Cluster Analysis Based on Molecular Level

Cluster analysis based on molecular level was employed to calculate the Euclidean distances among four rice genotypes and the dendrogram was constructed using these values as indicated in Figure 3. This dendrogram based on these values divided the four rice genotypes into two main clusters. The first cluster involved the promising line GZ6296-12-1-2-1-1 that recorded high values of chlorophyll content, panicle length, hulling %, milling %, and more tolerance to salinity according to the results in Table 1 and Figure 4. The rice genotype in this group is intrigued by rice breeders as a benefactor for breeding salinity tolerance. Whereas the second cluster involved the other three rice genotypes; Giza 179, Giza 178, and GZ1368-5-S-4 recorded the same values of salinity tolerance (Table 1 and Figure 4). The second cluster was divided into two sub-groups, the first group included Giza 179 which gave low values of growth duration and plant height and high values of panicles per plant, panicle length, 1000-grain weight, and grain yield. The second sub-group included Giza 178 and GZ1368-5-S-4, which exhibited identical values for the majority of the studied traits. The clustering genotypes were generally divided based on their origin and hereditary and summarized in previous reports (Ramadan *et al.*,

2017; Mazal, 2021 and Anis *et al.,* 2022). According to Bekis *et al.,* (2021), understanding genetic divergence and cluster distance across rice genotypes is necessary for genetic improvement, which is crucial for increasing crop productivity and production.

This study was employed for screening of salinity tolerance in four Indica-Japonica rice genotypes. A total of four SSR markers were reported to be linked with salinity tolerance genes which were screened among the four selected rice genotypes. Genotypic data showed that the four markers (RM212, RM9, RM493, and RM23) created polymorphic alleles among the considered genotypes and revealed diverse levels of polymorphism. The number of alleles varied from 2 to 11 alleles/locus and 23 amplified alleles were found to be polymorphic with an average of 5.75. The polymorphic alleles ranged from 2 to 10 with an average of 5. The polymorphic alleles play important roles in variety differentiation, diversity characterization, and conservation of potential parents (Pradhan, 2016 and Singh et al., 2016). Polymorphism information content (PIC) values reflection of allele diversity and frequency among genotypes also varied from one locus to another and provide an estimate of the discriminating power of the marker (Nagy et al., 2012). PIC value among the polymorphic markers varied from 0.352 for RM212 to 0.714 for RM9 with an average of 0.454. The identified polymorphism reflects the amount of diversity among the tested genotypes and thus the possibility of genetic improvement using such a set of genotypes in breeding programs since genetic diversity is the prerequisite for effective such programs.

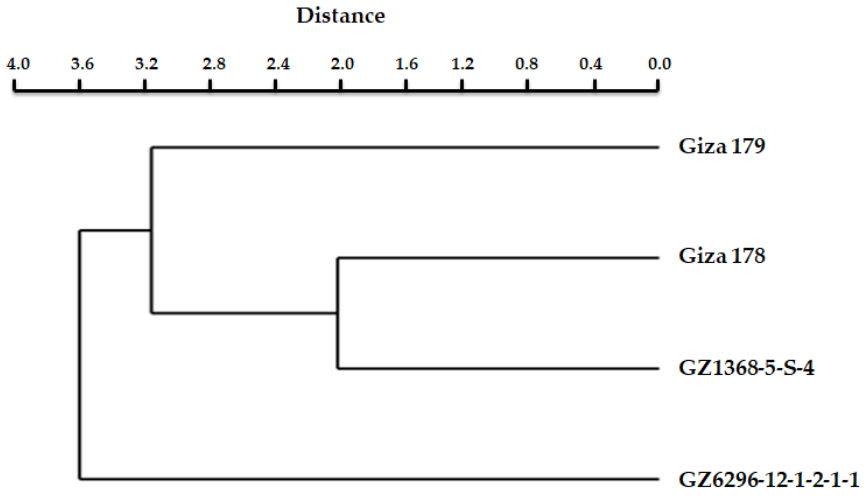

Figure 3: Dendrogram explaining the genetic relationships among tested rice genotypes using SSR markers employing the UPGMA method.

Table (6). Summary of molecular analysis for tested genotypes using SSR markers.

No	SSR primer	No. of alleles	No. of polymorphic alleles	PIC values
1	RM212	11	10	0.352
2	RM9	7	5	0.714
3	RM493	2	2	0.375
4	RM23	3	3	0.375
Total		23	20	1.816
Average		5.75	5	0.454

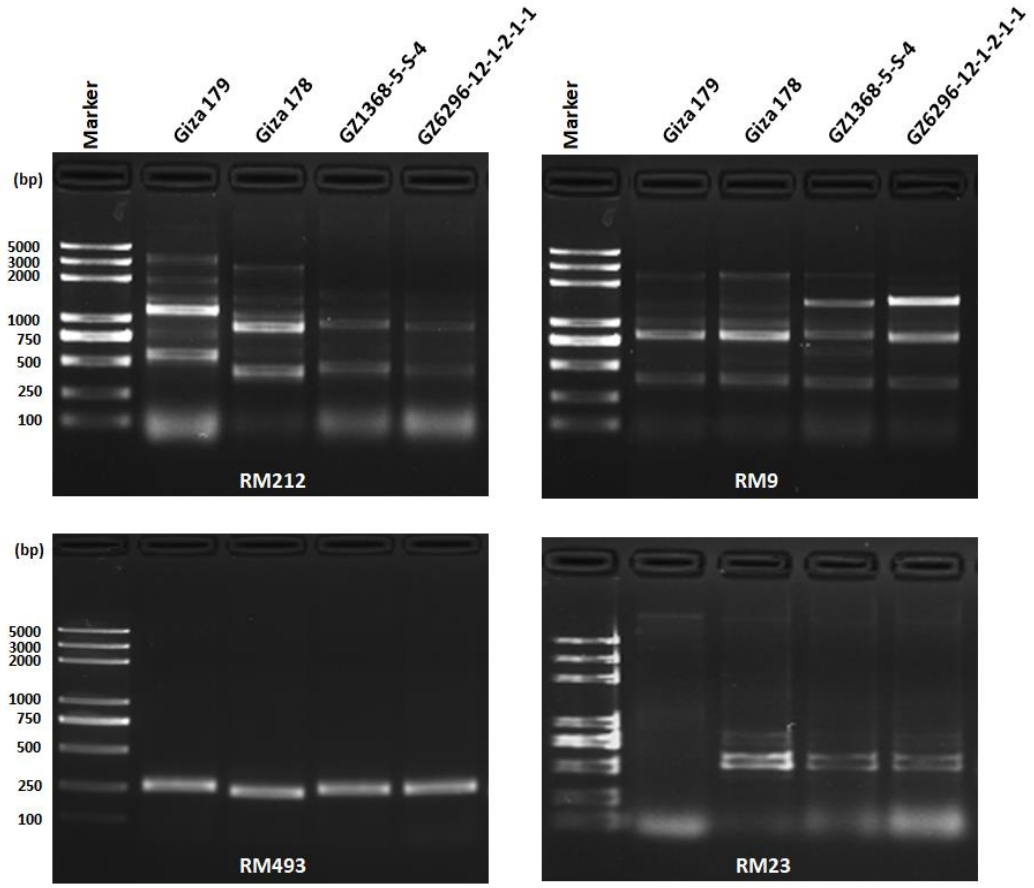

Figure 4: Banding pattern for four rice genotypes amplified by RM212, RM9, RM493 and RM23 markers.

Conclusion

The results showed that all the basic parents IR1515-31, BG94-2 AC1225, Hua Lien Yu 202, GZ1368-5-S-4, and GZ6296 contributed to producing the new variety Giza179. The analysis of variance showed highly significant differences for all the studied traits under saline soil conditions except root length and milling percentage. The main performance showed that the salinity conditions at the Lysimeter site negatively affected the yield trait for all rice genotypes compared with normal soil. The phenotypic coefficient of variability (PCV%) was greater than the genotypic coefficient of variability (GCV%) in all genotypes for all traits over two years. This means that most of the PCV% was influenced by environmental factors and cultural practices. Twelve SSR markers were identified, and association analysis revealed that the markers RM1212, RM9, RM493, and RM23 had the highest PIC values.

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تحسين أصناف الأرز المقاومة للملوحة باستخدام التربية التقليدية والجزيئية لتعزيز الإنتاجية

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تعتبرالملوحة هي أحد القيود الرئيسية لإنتاج الأرز المستدام، وتطوير صنف أرز جديد يتمتع بتحمل متأصل ضد هذه الضغوط غير الحيوية الرئيسية ويساعد في تحقيق زيادة مستدامة في إنتاج الأرز في ظل ظروف غير مواتية. أجريت الدراسة الحالية لتطوير جينات أرز تتحمل الضغوط غير الحيوية بخلفية وراثية لصنف الأرز التجارى المحسن جيزة ١٧٩ عن طريق التهجين الهرمي والمعلمات الجزيئية SSR. أظهرت النتائج أن جميع الآباء الأساسيين IR1515-31 وBG94-2 و AC1225و Hua Lien Yu 202 و GZ1368-5-S-4و GZ6296 ساهموا في إنتاج الصنف الجديد جيزة ١٧٩. أظهر تحليل التباين اختلافات كبيرة للغاية لجميع الصفات المدروسة في ظل ظروف التربة المالحة باستثناء طول الجذر ونسبة تصافى التبيض. أظهر الأداء الرئيسي أن ظروف الملوحة لها تأثير سلبي على صفة المحصول لجميع جينات الأرز مقارنة بالتربة العادية. كان معامل التباين الظاهري (PCV%) أعلى من معامل التباين الجيني (GCV%) في كلا الموسمين في جميع الأنماط الجينية لجميع الصفات، مما يشير إلى أن الجزء الأكبر من PCV% كان أكثر مساهمة نتيجة الظروف البيئية والممارسات الزراعية. أشارت اثنا عشر معلم جزيئى SSR ونتائج تحليل الارتباط إلى أن المعلمات الجزيئية RM1212 و RM9و RM493و RM23كانت ذات قيمة عالية من PIC .