J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 9 (2): 61 - 66, 2018

Molecular Evaluation of some Hybrid Rice Genotypes Using Microsatellite (Ssr) Marker under Normal and Saline Soil Conditions

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ABSTRACT

Eleven hybrid rice combinations were produced by using three cytoplasmic male sterility lines and seven restorers (line x tester mating design) evaluated for agronomic and yield characters under normal and salt soil conditions.The genotypes, Pusa 6A; Giza 178; GZ5121; IR69625A; IR69625A x PR1; Pusa 6A x Giza 178; IR69625A x GZ5121 and IR69625A x PR3 gave the lowest reduction for yield under saline soil condition. However, the genotypes IR69625A x GZ5121 (3.34 t./fed.) and IR69625A x PR3 (3.27 t./fed.) gave the highest yield under saline soil condition if compared with Giza 178 (2.94 t./fed.).Simple sequence repeat (SSR) markers were used to detect possible specific marker to be utilized in the hybrid rice future breeding programs for salt tolerance. However, SSR markers; RM21, RM302 and RM315 generated two (140, 170 bp), three (120, 170, 350 bp) and five DNA fragments (110, 130, 170, 180, 190 bp), respectively. These band can be used as positive or negative specific DNA band on the basis of their appearance or disappearance in the different gene types under study. It is of great interest to mention that the three DNA bands, 140 bp (RM21), 120 bp (RM302) and 190 bp (RM315) are considered as positive specific marker for salt tolerance, while they were appeared in the tolerant genotype (Giza 178) and other hybrid rice genotypes. These salt tolerant specific bands were found in the next genotypes; Pasua 6A x Giza 178, IR68902A, IR68902A x PR1, IR68902A x PR2, IR68902A x PR3, GZ6296R and Giza 182 may be due to heterosis effect, the origin or salt tolerant.Based on phylogenetic tree using similarity index, Pusa 6A x Giza 178, Pusa 6A x PRI, Pusa 6A x PR3 genotypes were found in one cluster and were closely related to the tolerant genotype Giza 178. On the other hand, phylogenetic tree showed that other four clusters were separated and proved that the genotypes PR2; PR3; GZ5121R; IR69625A x PR2; IR69625A x PR3 and IR69625A x GZ5121R were closely related to each other on the basis of their ability to tolerate salt stress.

Keywords: Hybrid rice, genetic diversity, salt stress, microsatellite marker, SSR.

INTRODUCTION

Rice is one of the most important food crops for consumes and exported. The national yield in the year of 2011 was 9.59 t./ha. (RRTC, 2012).

Environmental stresses such as low water availability, and salinity were affected on agricultural systems and represent major limitations to the yield and quality of rice and other crops. Salinity is a major abiotic stress affecting crops in Egypt and throughout the world. More than 800 million hectares of land are salt affected globally, accounting for more than 6% of total land area (Munns and Tester, 2008). Egypt is one of the countries that suffer severe salinity problems. Over 33% of the cultivated land which comprises only 3% of total land area in Egypt is already saline (Ghassemi *et al*., 1995). Egyptian lands suffer from salinity problem, whereas rice is more common crop under area affected by salt stress in Egypt. Rice grain yield under target area still low as compared to normal soil. Thereby, increasing grain yield of rice in those area is badly needed for food security and poverty alleviation (El-Mowafi, 1994 and Zayed *et al*., 2015).

DNA techniques allow the assessment of a theoretically unlimited number of polymorphic marker loci (Nguyen *et al*., 2004). Molecular markers were used to evaluate the extent of genetic variability, among these markers Simple Sequence Repeat (SSR) is the marker of choice for many genetic analyses in hybrid rice. SSR markers have a number of advantages, such as the high level of polymorphisms, locus specificity, co-dominance, reproducibility convenience through using PCR and random distribution throughout the genome (Powell *et al*., 1996). It is ideal for marker assisted breeding (Deric *et al*., 2005), genetic mapping (Ramsay *et al*., 2000). Finally SSR marker is technically efficient, cost-effective to use and are available for hybrid rice (Wang *et al*. 2006; and Al-Ibrahim, 2012). The present study aimed to develop marker associated with salt tolerance in hybrid rice using SSR marker to be utilized in the future breeding for salt tolerance in hybrid rice program.

MATERIALS AND METHODS

The present investigation was conducted in Biotechnology Laboratory of Genetic Department, Faculty of Agriculture, Kafrelsheikh University during 2015 and 2016 of rice growing seasons. Hybrid rice genotypes used in this study are shown in Table (1): three females (CMS lines), seven restorer testers (R) and their 11 F_1 hybrid combinations were tested under normal and saline conditions using L x T mating. Three parents (Giza 178; GZ6296R and Giza 182) were used as scale of salinity tolerance. Genotypes were selected from 21 genotypes based on their tolerance/sensitivity to salinity stress. The trials were conducted in a randomized block design (RBD), using three replicates under two locations [normal Sakha EC=1.5 dS/m] and [saline El-Sirw EC = 7.5-10.3 (dS/m)] in 2012 growing season. Evaluted data were on ten randomly plants in each location in replications for; days to flowering (day); plant height (cm); tillers plant^{-1}; filled grains panicle^{-1}; 1000-grain weight (g) and grain yield (t./fed.). These traits and reduction percentage (R%) = are most likely affected by salt stress and soil conditions.

SSR analysis

DNA was isolated by CTAB method (Doyle and Doyle, 1990). Three SSR primers were used in this study, sequences of used primers were illustrated in Table 2.

PCR amplification was performed in a total volume of 20 µl containing 2.5 µl 25 mM $MgCl_2$, 2µL 2.5 mM dNTPs, 2 µl 10 PMol primer, 1 µl 50 ng of genomic DNA and 0.2 µl *taq* DNA polymerase (5 units/µl).

PCR amplification was applied for one cycle at 95°C for 5 min, then 35 cycles were performed as follows: 1 min at 95°C for denaturation, 30 sec. at 55°C to 58°C for SSR and 45 sec. at 72°C for extension. Reaction was incubated at 72°C for 7 min then at 4°C.

The products were separated by electrophoresis using 2% agarose gel in 0.5 x TBE buffer against 100 bp DNA ladder. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on gel documentation. Results of simple sequence repeate (SSR) application were comparable to each other and DNA fragments were scored as a binary data, where (1) means presence and (0) means absence. Data were used to estimate genetical similarity on the basis of number of shared amplifications product. Phylogenetic tree based on Jaccard method for tested SSR primers and similarity index based on Jaccard methods on SSR primers were used (Hammer *et al*., 2001).

Table 1. Cytoplasmic male sterile and restorer lines in this research.

Genotype	Cytoplasmic source	Origin
CMS lines (female):		
Pusa 6A	WA (Aromatic CMS)	India-Egypt
IR69625A	WA (Hybrid rice program)	IRRI
IR68902A	WA (Aromatic CMS)	IRRI
Restorer lines		
PR1	New aromatic restorer developed by HRB program in Egypt.	Egypt
PR2	New aromatic restorer developed by HRB program in Egypt	Egypt
PR3	New aromatic restorer developed by HRB program in Egypt	Egypt
Giza 178R	Restorer and tolerance to salinity.	Egypt
Giza 182	Restorer.	Egypt
GZ5121R	Restorer and tolerance to salinity.	Egypt
GZ6296R	Restorer.	Egypt

Table 2. Sequence were as follweing SSR primers.

Primer name	Forwarded	Reverse
RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
RM302(CH1)	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
RM315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG

RESULTS AND DISCUSSION

Field screening

Mean performance of three CMS lines (A lines), seven restorers and 11 F_1 hybrids for six of agronomical and yielding characters under normal (N) and sailt stress (S) conditions, more over to reduction percentage (R%) was shown in Tables 3 and 4. Solt stress caused delay in the groweing of some genotypes. Tolerance to solt stress differs from one genotype to other. The most affected hybrids were; Pusa 6A x PR1, Pusa 6A x PR2 and Pusa 6A x PR3 with 7.34; 7.34 and 7.33 delay days in flowering, under normal (N) and salt stresses. The days required by genotype IR68902A (106.67). On the obosit side, the best genotype for early was GZ6296 (92.34 day) under normal condition. Plant height was sharply significantly affected by salinity. Reduction in plant tall caused by salt stress differed from one genotype to other. However, the reduced ranged from 2.46% to 20.13% for the genotypes GZ5121R and IR68902A. The most affected genotype was IR68902A (20.13%). From another view, the lowest reduction and tolerant genotypes were GZ5121 (2.46%) and Pusa 6A x PR3 (2.47%). Similar results were obtained by El-Mowafi (1994). For tillers per plant, the hybrid genotypes, IR69625A (6.62%), Pusa 6A (10.24%) and IR69625A x GZ5121 (11.05%) showed the smallest reduction for tillers per plant.

For number of filled grains panicle^{-1}, the greatest mean values under salt stresses registered by the genotypes Pusa 6A x Giza 178R (176.25), after that Pusa 6A x PR3 (174.97) and Pusa 6A x PR1 (171.38). These results may be for genotype and hybrid vigor effects. However, the hybrid rice combination IR69625A x GZ5121 gave lessens reduction in number of filled grains per panicle (22.62%), but for restorers; Giza 178 (17.75%) and GZ5121 (19.17%).

The results showed that 1000-grain weight (g) of 21 genotypes were significantly depressed under salt stresses if comparable with normal soil. Salt stress had different effects according to the genotype. Under both normal and salt stresses, hybrids which had the biggest mean values were; IR69625A x PR1 (28.46 and 26.69 g); IR69625A x PR2 (28.19 and 26.03 g); IR69625A x PR3 (28.82 and 26.78 g); Pusa 6A x PR3 (28.09 and 25.32 g); PR3 (29.82 and 26.32 g); PR1 (28.80 and 25.21 g) and PR2 (28.66 and 25.22 g). These hybrids showed superiority in 1000-grain weight over all other observed genotypes. Meanwhile, the lowest reduction (R%) in 1000-grain weight was tested in the genotypes; IR68902A x PR1 (5.00%), IR69625A x GZ5121R (6.10%), IR68902A x PR2 (6.47%), IR69625A x PR1 (6.23%), and IR69625A x PR3 (7.08). The smallest reduction in 1000-grain weight under salt stresses for some plants reflected their tolerance to salt stress. The salt stress on the grain yield was highly significant for all genotypes.

In addition, the bigger degrees of salinity caused a sharp decrease in grain yield of all genotypes. The followed hybrids; IR69625A x PR3 (5.81 ton/fed); Pusa 6A x PR3 (5.75 t./fed); Pusa 6A x PR2 (5.69 t./fed.) and Pusa 6A x PR1 (5.68 t./fed.) gave the greatest grain yield under normal soil. On another side, under salt stresses, the highest grain yield (t./fed.) was obsearved by the hybrids; IR69625A x GZ5121 (3.34 t./fed.); in the second roof was IR69625A x PR3 (3.27 t./fed.) and Pusa 6A x PR3 (3.25 t./fed.). The stress percentage in grain yield per plant (t./fed.) differance from one genotype to another. The greatest reduction was obsearved by IR68902A (56.21%) and Pusa 6A x PR2 (51.14%). On another view, the smallest reductions were for GZ5121 (30.53%); Giza 178R (34.66%); Pusa 6A (35.58%); IR69625A (38.38%) and IR69625A x GZ5121 (39.63%). These results are in agreement with the results of El-Mowafi (1994), and Soltan (2007), who found that plant

phenotype is a product of genotype and environment, these leading environmental factors to modify the tolerance of plant. Salinity and other environmental effects interact in several ways that may obscure inheritance studies of salt tolerance.

Table 3. Means of hybrid rice genotypes for agronomical characters under normal and salt stresses.

Genotypes	Days to heading (days)			Plant height (cm)			Tillers plant^{-1}		
	N	S	R%	N	S	R%	N	S	R%
CMS (Female line)									
Pusa 6A	104.66	106.66	-1.91	95.30	89.66	5.92	21.20	19.03	10.24
IR69625A	101.33	102.66	-1.31	107.60	97.00	9.85	20.70	19.33	6.62
IR68902A	106.66	108.33	-1.57	111.00	88.66	20.13	19.00	15.56	18.11
Restorer									
PR1	101.66	102.66	-0.98	115.60	100.33	13.21	16.10	14.13	12.24
PR2	101.33	102.66	-1.31	119.30	101.66	14.79	17.70	13.56	23.39
PR3	101.00	100.66	0.34	118.00	104.33	11.58	17.40	15.43	11.32
Giza 178R	101.66	103.00	-1.32	99.00	95.00	4.04	26.50	21.93	17.25
Giza 182R	98.66	98.66	0.00	96.00	84.00	12.50	20.20	16.13	20.15
GZ5121R	104.66	106.66	-1.91	95.00	92.66	2.46	22.90	20.33	11.22
GZ6296R	92.33	90.00	2.52	85.00	75.00	11.76	23.80	17.96	24.54
Hybrid combinations									
Pusa 6A x PR1	94.33	101.66	-7.77	109.30	96.33	11.87	23.30	20.26	13.05
Pusa 6A x PR2	95.33	102.66	-7.69	108.30	103.33	4.59	20.10	17.63	12.29
Pusa 6A x PR3	96.00	103.33	-7.64	108.00	105.33	2.47	21.60	18.46	14.54
Pusa 6A x Giza 178R	100.33	102.00	-1.66	105.00	99.66	5.09	26.30	22.96	12.69
IR69625A x PR1	99.00	102.33	-3.36	116.60	105.66	9.38	23.00	18.90	17.83
IR69625A x PR2	99.00	100.33	-1.34	115.30	104.66	9.23	24.90	19.53	21.57
IR69625A x PR3	99.66	101.33	-1.68	111.00	105.33	5.11	26.02	21.70	16.54
IR69625A x GZ5121R	10.66	105.66	-1.93	105.60	96.33	8.78	25.60	22.77	11.05
IR68902A x PR1	103.66	106.00	-2.26	112.60	102.00	9.41	19.80	16.10	18.69
IR68902A x PR2	103.33	105.66	-2.25	111.60	103.66	7.11	20.10	16.67	17.06
IR68902A x PR3	102.66	104.66	-1.95	109.60	104.66	4.51	20.50	17.67	13.80

N = Normal soil in Sakha, S = Saline soil in El-Sirw R% = Reduction percentage

Table 4. Mean performance of hybrid rice genotypes for yielding under normal and salt stresses.

Genotypes	Filled grains Per panicle			1000-grain weight (g)			Grain yield (t./fed.)		
	N	S	R%	N	S	R%	N	S	R%
CMS (Female line)									
Pusa 6A	176.30	121.01	31.36	25.52	21.95	13.99	3.57	2.30	35.57
IR69625A	133.06	100.20	24.69	27.68	23.49	15.14	3.62	2.23	38.40
IR68902A	143.56	87.72	38.90	22.56	17.61	21.99	3.22	1.41	56.21
Restorer									
PR1	195.10	149.61	23.32	28.80	25.21	12.47	4.23	2.51	40.66
PR2	184.90	147.41	20.28	28.66	25.22	12.03	4.27	2.39	44.03
PR3	194.00	152.89	21.19	29.82	26.32	11.74	4.27	2.59	39.34
Giza 178R	140.66	115.70	17.75	22.05	20.01	9.25	4.50	2.94	34.67
Giza 182R	132.96	94.99	28.56	27.07	23.13	14.56	4.25	2.22	47.77
GZ5121R	126.43	102.20	19.17	26.15	24.24	7.30	4.16	2.89	30.53
GZ6296R	158.50	119.74	24.45	27.79	24.26	12.70	4.24	2.68	36.79
Hybrid combinations									
Pusa 6A x PR1	233.23	171.38	26.52	27.47	22.44	18.31	5.68	3.05	46.30
Pusa 6A x PR2	226.33	164.11	27.49	27.37	22.38	18.23	5.69	2.78	51.14
Pusa 6A x PR3	242.80	174.97	27.94	28.09	25.32	9.86	5.75	3.25	43.48
Pusa 6A x Giza 178R	250.83	176.25	29.73	26.55	23.61	11.07	5.59	3.23	42.22
IR69625A x PR1	207.03	154.72	25.27	28.46	26.69	6.23	5.56	3.22	42.09
IR69625A x PR2	201.20	146.02	27.43	28.19	26.03	7.66	5.67	3.24	42.86
IR69625A x PR3	203.40	150.37	26.07	28.82	26.78	7.08	5.81	3.27	43.18
IR69625A x Gz5121R	170.96	132.30	22.61	27.06	25.41	6.10	5.53	3.34	39.60
IR68902A x PR1	218.40	138.16	36.74	25.18	23.92	5.00	5.08	2.60	48.82
IR68902A x PR2	217.53	142.51	35.08	25.18	23.55	6.47	5.19	2.65	48.94
IR68902A x PR3	218.06	143.81	34.05	26.72	24.04	10.03	5.43	2.76	49.17

N = Normal soil in Sakha, S = Saline soil in El-Sirw R% = Reduction percentage

SSR analysis

Three SSR primers were used in this investigation to evaluate the genetic diversity among the 21 hybrid rice used herein. These primers revealed a total of 10 alleles ranging from two alleles using primer RM21 to five alleles using primer RM315 (Table 5 and Fig. 1). Polymorphic bands ranged between 140 and 170 bp generated by RM21 primers, genotypes around 140 bp, which considered as a positive marker for salt stress in hybrid rice genotypes. RM302 primer appeared three polymorphic bands ranging between 120 to 350 bp, one of them was found in the tolerant genotypes with size around 120 bp, which is considered as a positive marker for salt stress.

The dendrogram depend on SSR marker data successfully discriminated six main clusters for salinity tolerance. Cluster 1 includes; IR68902A; IR68902A x PR1; in the same time; Giza178; Giza182 and GZ6269 was in sub cluster. Cluster 2 included two crosses; IR68902A x PR2 and IR68902A x PR3; Cluster 3 contains Pusa 6A x Giza178; Pusa 6A x PR1 and PR1. Thus, cluster 4 contain Pusa 6A only. Cluster 5 contains two genotypes; Pusa 6A x PR3 and PR3. Thus, cluster 6 contains only two genotypes; Pusa 6A x PR2 and PR2. Higher similarity (100%) was appeared in Giza182R and Giza178R, similarity 50 % in Pusa 6A x Giza178R and IR68902A x PR1, similarity 25 % in Pusa 6A x PR1 and PR1, similarity 20% in IR68902A, IR68902A x PR2 and IR68902A x PR3.

Similarity index was shown in Table (6) and Figure (2), showed similarity that may be due to its origin and heterosis effect or salt tolerant genotypes compared with Giza 178 which highly diverged and distance from GZ6296. similarity was ranged from 0 to 1.

Three primers markers used with twelve genotypes of hybrid rice shown DNA polymorphism in Table (7) and Fig. (3).

Fig.1. PCR amplification profile generated from genomic DNA of 15 hybrid rice genotypes with SSR primers, RM21, RM302 and RM315.

Note: M-marker = 100 bp, DNA ladder 1- Pusa 6A, 2- Pusa 6A x G.178, 3- Pusa 6A x PR1, 4- PR1, 5- Pusa 6A x PR2, 6- PR2, 7-Pusa 6A x PR3, 8- PR3, 9- IR68902A, 10-IR68902A x PR1, 11- IR68902A x PR2, 12- IR68902A x PR3, 13- Giza 178, 14- GZ6296R and Giza 182.

Table 5.Banding pattern of DNA Polymorphism for fifteen genotypes with three SSR Primers.

Gent Primers	Band size	Band	Pusa 6A	Pusa6A X G178	Pusa6A X pR1	PR1	Pusa6A X PR2	PR2	Pusa6A x PR3	PR3	IR68902A	IR68902A x PR1	IR68902A x PR2	IR68902A x PR3	Giza 178	GZ6296 R	Giza 182
RM 21	140	1	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1
	170	2	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
RM302	120	1	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1
	170	2	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0
	350	3	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0
RM315	110	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	130	2	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	170	3	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0
	180	4	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	190	5	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1

Gent = Genotypes

Table 6. Similarity index based on Jaccard similarity for salinity tolerant

Gent.	Pusa6A	Pusa6A x G.178	Pusa6A x PR1	PR1	Pusa6A x PR2	PR2	Pusa6A X PR3	PR3	IR68902A	IR68902A x PR1	IR68902A x PR2	IR68902A x PR3	Giza 178	GZ6296 R	Giza 182
Pusa 6A	1.00														
Pusa 6A x G.178	0.25	1.00													
Pusa 6A x PR1	0.33	0.67	1.00												
PR1	0.33	0.67	1.00	1.00											
Pusa 6A x PR2	0.33	0.00	0.00	0.00	1.00										
PR2	0.25	0.00	0.00	0.00	0.67	1.00									
Pusa 6A x PR3	0.00	0.00	0.00	0.00	0.00	0.20	1.00								
PR3	0.00	0.00	0.00	0.00	0.00	0.20	1.00	1.00							
IR68902_A	0.25	0.20	0.00	0.00	0.25	0.20	0.20	0.20	1.00						
IR68902A x _PR1	0.00	0.50	0.25	0.25	0.00	0.00	0.20	0.20	0.50	1.00					
IR68902A x PR2	0.25	0.20	0.00	0.00	0.25	0.20	0.00	0.00	0.50	0.20	1.00				
IR68902A x PR3	0.00	0.20	0.00	0.00	0.00	0.00	0.20	0.20	0.20	0.20	0.50	1.00			
Giza178	0.00	0.50	0.25	0.25	0.00	0.00	0.00	0.00	0.20	0.50	0.20	0.20	1.00		
GZ6296R	0.00	0.20	0.00	0.00	0.00	0.00	0.20	0.20	0.20	0.20	0.20	0.50	0.50	1.00	
Giza 182	0.00	0.50	0.25	0.25	0.00	0.00	0.00	0.00	0.20	0.50	0.20	0.20	1.00	0.50	1.00

Gent = Genotypes

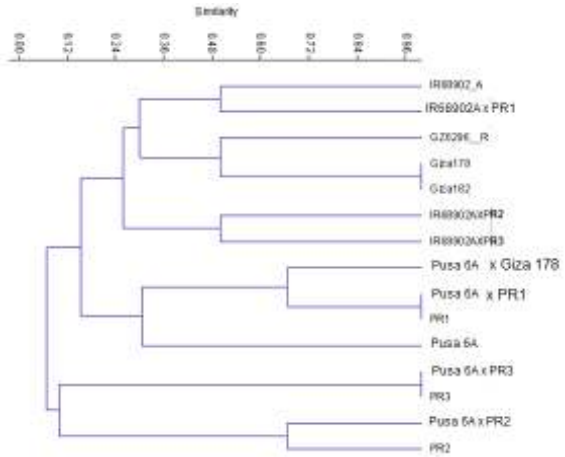

Fig. 2. Phylogenetic tree based on Jaccard method similarity of primers tested for salinity tolerance in rice.

These markers generated two, three and five DNA fragments with different molecular sizes among the highest and lowest tolerant parents. It was clear that, RM21 marker generated two fragments with expected sizes of 140 and 170 bp in tolerant and sensitive, RM302 marker generated three fragments with expected sizes of 120, 170 and 350 bp among the highest and lowest tolerant. RM315 marker appeared five fragments with expected molecular sizes of 110, 130, 170, 180, and 190 bp among the studied genotypes. In addition, another fragent of RM 21 with molecular size of 140 bp was identified in the four restorers PR1, Giza178,GZ6296 and Giza182. However, 170 bp band was identified in two restorer lines PR2 and PR3. The SSR marker RM302 apeared three different bands, the first one with molecular size of 120 bp was generated in the three restorer lines PR1, Giza178 and Giza182 and the CMS line IR69625A, the seconed one with molecular size of 170 bp was generated in PR2 and the three hybrids IR69625A x GZ5121R, IR69625A x PR1 and IR69625A x PR2, while the third band of 350 bp was identified in three restorer lines GZ5121, PR3 and GZ6296 and the hybrid IR69625A x PR3. RM315 generated four DNA fragments with molecular sizes of 110, 170, 180 and 190 bp. The first band was appeared in PR1 and PR2. The second band was appeared in PR3 and the three hybrids IR69625A x GZ5121R, IR69625A x PR2 and IR69625A x PR3. The third band was identified in the restorer line GZ5121R. the last band was generated in the restorer lines Giza178, Giza182 and GZ 6296R and one hybrid IR69625A x PR1 . These generated bands could be used as specific DNA markers deppending on appearance or disappearance in the different rice genotypes under the current study.

Table 7. Banding pattern of DNA Polymorphism for salinity tolerance.

Primers	band size	Band	IR69625A	IR69625A x GZ5121R	GZ5121R	IR69625A x PR1	PR1	IR69625A x PR2	PR2	IR69625A x PR3	PR3	Giza 178	GZ6296R	Giza 182
RM21	140	1	0	0	0	0	1	0	0	0	0	1	1	1
	170	2	1	1	0	1	0	1	1	1	1	0	0	0
RM302	120	1	1	0	0	0	1	0	0	0	0	1	0	1
	170	2	0	1	0	1	0	1	1	0	0	0	0	0
	350	3	0	0	1	0	0	0	0	1	1	0	1	0
RM315	110	1	0	0	0	0	1	0	1	0	0	0	0	0
	130	2	0	0	0	0	0	0	0	0	0	0	0	0
	170	3	0	1	0	0	0	1	0	1	1	0	0	0
	180	4	0	0	1	0	0	0	0	0	0	0	0	0
	190	5	1	0	0	1	0	0	0	0	0	1	1	1

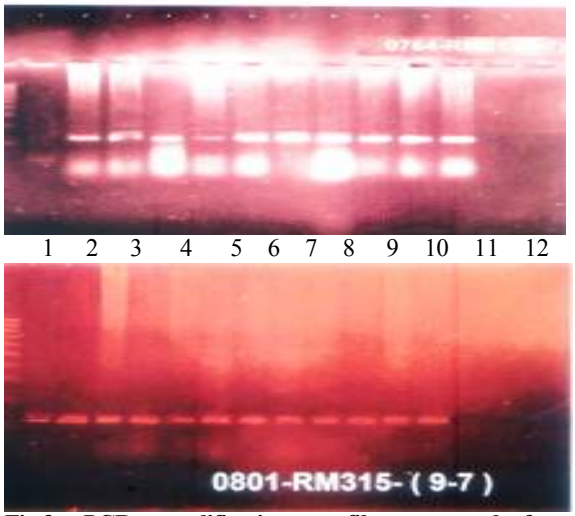

Fig.3. PCR amplification profile generated from genomic DNA of twelve hybrid rice genotypes with SSR primers, RM21 and RM315.

Note: M-Marker = 100 bp, DNA ladder, 1: IR69625A, 2: IR69625A x GZ5121R, 3: GZ5121R, 4: IR69625A x PR1, 5: PR1, 6: IR69625A x PR2, 7: PR2, 8: IR69625A x PR3, 9: PR3, 10: Giza 178, 11: GZ6296R and 12: Giza 182.

As shown in Table (8) and Figure (4), the cluster analysis based on SSR markers data successfully discriminated the studied genotypes for salinity tolerance. Four main clusters were found; cluster 1 included two rice genotypes IR69625A x PR1 and IR69625A. Cluster 2 included two rice genotypes IR69625R x PR3 and PR3. However, PR1, IR69625A x GZ5121R and IR69625A x PR2 was located as sub cluster. Cluster 3 included only one restorer line GZ6296R. However, the restorer lines Giza 178, PR1 and Giza 182 were located as sub cluster. Meanwhile, cluster 4 included GZ5121R and showed high similarity of 100% between Giza182 and Giza178, similarity of 50% between IR69625A, PR1 and GZ6296R and similarity of 20% for IR69625A x PR1. The obtained similarity index suggested that they are salt tolerant.

The genotypes; PR2 ; PR3; GZ5121R ; IR69625A x PR2; IR69625A x PR3 and IR69625A x GZ5121which highly diverged and distance from GZ6296 with similarity of 0 from to 1 may be due to heterosis effect , the origin or salt tolerant.

Similar results were obtained by Al-Ibrahim (2012) and Hakim *et al*., (2014), who suggested that some of these markers can be linked to stress tolerant genes of tolerant genotypes that can be transferred to good yield but sensitive cultivar(s) through marker assisted selection (MAS) (Zhong *et al*., 2006).

Table 8. Phylogenetic tree based on Jaccard method similarity for salt tolerant genotypes.

Gent.	IR69625A	IR69625A x GZ5121R	GZ5121R	IR69625A x PR1	PR1	IR6962A x PR2	PR2	IR69625A X PR3	PR3	Giza 178	GZ6296R	Giza 182
R69625A	1.00											
IR69625A x GZ5121R	0.20	1.00										
GZ5121R	0.00	0.00	1.00									
IR69625A x PR1	0.50	0.50	0.00	1.00								
PR1	0.20	0.00	0.00	0.00	1.00							
IR69625A x PR2	0.20	1.00	0.00	0.50	0.00	1.00						
PR2	0.20	0.50	0.00	0.50	0.20	0.50	1.00					
IR69625A x PR3	0.20	0.50	0.25	0.20	0.00	0.50	0.20	1.00				
PR3	0.20	0.50	0.25	0.20	0.00	0.50	0.20	1.00	1.00			
Giza178	0.50	0.00	0.00	0.20	0.50	0.00	0.00	0.00	0.00	1.00		
GZ6296R	0.20	0.00	0.25	0.20	0.20	0.00	0.00	0.20	0.20	0.50	1.00	
Giza182	0.50	0.00	0.00	0.20	0.50	0.00	0.00	0.00	0.00	1.00	0.50	1.00

Gent. = Genotype

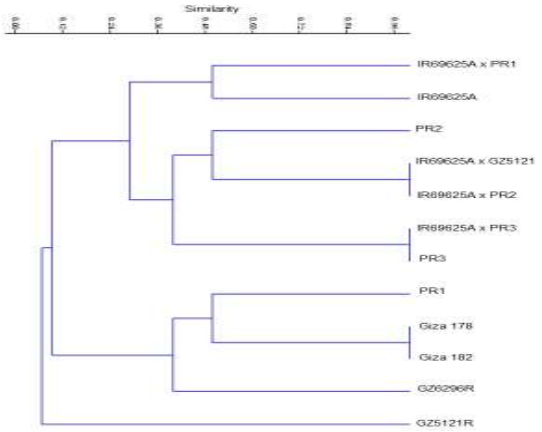

Fig. 4. Phylogenetic tree depend on Jaccard method similarity for salinity tolerance genotypes of rice hybrids.

Finally, data showed that simple sequance repeat (SSR) primers could be used as a marker assisted selection (MAS) in rice for salinity breeding programs.

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إستخدام المعلمات الجزيئية لتقييم بعض طرز الأرز الهجين تحت ظروف الأراضى العادية والملحية

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تم هذا البحث على ١١ طراز وراثى أنتجت من تهجين ثلاث سلالات عقيمة الذكر سيتوبلازميا مع سبع سلالات معيدة للخصوبة أو ملقحات منها ثلاث آباء للمقارنة واستخدم فيها نظام التزاوج السلالة ×الكشاف (L x T) وتم زراعة التجربة تحت ظروف الاراضى العادية بسخا والملحية بالسرو .والهدف من تلك الدراسة تحديد بعض الادلة الجزيئية المرتبطة بتحمل الملوحة من عدمه مقارنة بالصنف المتحمل للملوحة جيزة ١٧٨ وربطها ببعض الصفات الحقلية والمحصول.وخلصت الدراسة إلى أن التراكيب الوراثية Pusa6 x Giza 178 A ,, Giza 178, GZ5121R, IR69625A, IR69625A x PR1, Pusa6A أعطت أقل معدل نقص بالنسبة لمعظم الصفات تحت الدراسة ، وأن الهجن IR69625A x PR3 (٣.٢٧٠ طن/ فدان) R69625A x GZ5121R (٣.٣٤٠طن/فدان). أعطت أعلى محصول تحت ظروف الاراضى الملحية مقارنة بالصنف جيزة178 (2.94 طن/فدان) . أظهر تحليل المعلمات الجزيئية (SSR) بإستخدام Specific primers (RM21, RM302, RM315) وجود عشرة أليلات تراوح وزنهم الجزيئى من 110-350 bp كما أظهر التحليل العنقودى ودليل التشابه أو القرابة باستخدام طريقة Jaccard بأن التراكيب الوراثية Pusa6Ax PR2 وIR69625A x PR2 ،IR69625A x PR3 ،Pusa6A x PR1,Pusa6A x Giza178, وA x PR3 IR69625A x PR3 ،IR69625A ،PR3 ،PR2 ،PR1 ،IR69625A x GZ5121R ،Pusa6 المتحمل للملوحة جيزة ١٧٨ وبالتالى يوجد علاقة أو ارتباط بالنسبة لصفة تحمل الملوحة .بينما التراكيب الوراثية IR68902A, IR68902A x PR1, IR68902A x PR2, IR68902A x PR3, GZ6296R, Giza 182أظهرت وجود تشابه بالصنف المقارن جيزة ١٧٨ كصنف متحمل للملوحة إلا أن هذا التشابه أو القرابة ربما ترجع إلى قوة الهجين أو المنشأ وليس لصفة تحمل الملوحة وبالتالى تحتاج هذه إلى دراسة أكثر لتوضيح هذه النتائج.