IDENTIFICATION OF SOME MOLECULAR AND BIOCHEMICAL MARKERS ASSOCIATED WITH SALINITY TOLERANCE IN RICE (*ORYZA SATIVA*, L.)

EL-REFAEE, Y. Z. [1], M.M. ASFOUR[2] AND A.E. DRAZ[1]

1- Rice Research & Training Center (RRTC) Sakha, Kafr El-Sheikh, Field Crop Research Institute, ARC, Giza, Egypt.

2- Environmental Studies and Research Institute, Menofiya University.

Abstract

Seven rice genotypes were used in this study. They were characterized by a reasonably wide range of variation according to their salt tolerance. They were exposed to saline and normal conditions. The biochemical markers analyses were also screened to detect the polymorphism through the saline tolerant and sensitive rice genotypes and to detect the quantitative traits for characterization and identify useful genes under saline conditions. SDS-PAGE showed that common protein bands of 66 KDa, 38 KDa and 20 KDa can be considered as markers associated with salt tolerance. Esterase isozyme analysis showed very weak and intermediate bands (No.2 and 4), respectively that can be considered as markers associated with salt tolerance. Peroxidase isozyme analysis showed two intermediate bands (No.4) and (No.10), which can be considered as markers associated with salt tolerance. Eight positive ISSR bands and five negative ISSR bands were recorded and can be considered as markers associated with salt tolerance.

Key words: rice, molecular and biochemical markers, salinity tolerance, genotype, polymorphism, protein, isozyme.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the agronomically and nutritionally most important cereal crops. It is a major source of food for more than 2.7 billion people and is planted on about one-tenth of the earth's arable land. It is the staple food and the largest source of energy for more than half of the world's population, most of them are in the developing countries. Besides its economic significance, rice is rich in genetic diversity in the form of thousands of land races and related wild species (Naghia *et. al.*, 2002).

Most of commonly cultivated rice genotypes at seedling and reproductive growth stages are particularly sensitive to root-zone salinity. More importantly though,

the yield components related to final grain yield are severely affected by salinity. For example, panicle length, spikelets per panicle and grain yield are significantly reduced by salt treatments (Khatun *et. al.*, 1995).

Using different molecular markers as a tool for genetic fingerprinting facilitate the identification and characterization differences in germplasm or identifying useful genes within the collected genetic resources or in segregant populations. In this regard, protein, isozyme and DNA analysis constitute important tools for genetic studies revealing the genetic diversity and relatedness among species, population and individuals.

Since each marker system has specific advantages and disadvantages, the choice of the marker system to be used is the most important decision. To compare the effectiveness, several comparisons among the different classes of PCR-based markers were carried out. For instance, Palombi and Damiano (2002) compared RAPD and SSR-markers to detect genetic variability in kiwifruits. Similarly, Corazza-Munes *et. al.*, (2002) and Archak *et. al.*, (2003) either compared the efficiency of RAPD, SSR and AFLP or used the different classes of markers for genotype identification. Recently, the efficacies of different classes of PCR based markers were also used to characterize barley and rice cultivars (Saker et al., 2005 and Virk *et. al.*, 2000).

Since salt tolerance has low heritability, phenotypic selection is difficult and progress through conventional breeding, although noticeable, has been slow. Conventional breeding will be enhanced greatly by biotechnological tools, allowing breeders to formulate more proficient breeding strategies (Collard and Mackill, 2007). There is still a gap between genomics and its application to breeding and although the possibilities are indefinite, a long way still needed to be accomplished (Xu et al., 2005). Recent advent of molecular markers, microsatellites or simple sequence repeats (SSRs) increased the hope to find out salt tolerant rice genotypes (Bhowmik *et. al.*, 2009).

The present study was undertaken to achieve the following objectives: a) Identification of one or more markers that could be linked to some important genes, which are responsible for salinity tolerance, and b) using Protein, isozyme and ISSR markers to identify the relationship between salinity tolerance traits and the molecular and or biochemical markers.

MATERIALS AND METHODS

Materials:

Seven rice cultivars were utilized in this study namely, Agami, Giza 159, GZ1368-S-5-4, Giza 178, Giza 177, Sakha102 and IR 29. The first four cultivars are considered to be salt tolerant and the last three ones are considered to be sensitive. The genotypes used were characterized by a reasonably wide rang of variation in their tolerance to salinity.

Methods:

Experimental field procedures:

Two experiments were conducted during 2010 growing season under two different conditions: Normal condition at the experimental farm of Rice Research &Training Center (Sakha, Kafr El-Sheikh, Egypt) and saline condition under Lysimeter (artificial salinization condition) to determine the varietal differences for salinity tolerance.

Normal condition:

The above mentioned seven rice genotypes utilized in this study were grown in the nursery during the third week of May. Thirty days after sowing, seedlings of each genotype was individually transplanted in the permanent field in a randomized complete block design (RCBD), with three replications, each replicate consisted of five rows for each genotype, five meters long and 20 cm apart between plants and rows. Weeds were chemically controlled by applying two liters of Saturn/feddan four days after transplanting. Nitrogen fertilizer was applied at 60 kg N/fed.

Lysimeter experiment:

This experiment was conducted in 2010 season to determine the varietal differences in salt tolerance under Lysimeter condition. The Lysimeter is concrete beds to 100 cm depth in three layers as follow: 60 cm clay at surface, 20 cm sand in the middle and 20 cm gravel in the bottom. In 2010 season, all the seven rice genotypes sown in the nursery during the third week of May were transplanted after 30 days to Lysimeter.

Adapted of 15 x 15 cm inter and intra row-spacing was used. Three replications were grown in a randomized complete block design. The materials were grown in Lysimeter under salinity level of 6000 ppm. Stock solutions of the essential elements were prepared using Sodium Chloride (NaCl) and Calcium Chloride (CaCl2) at the ratio of 2:1 respectively. Salinization, irrigation and drainage cycle were accurately controlled. The mean value of electrical conductivity (EC) of the irrigation water was 10.6 mm/cm at 25^{O}C for the salinity level. The plots were salinized 15 days after transplanting and salinization was fixed till harvesting. Plants were irrigated

every day by out pumping the salt solution from the tank, drainage was practiced every 48 hours through bottom outlets and water electrical conductivity (EC) was measured through the crop season.

Studied traits:

Data collected were: days to heading, plant height (cm), chlorophyll content (mg/ds), panicle length (cm), number of panicles per plant, tillers/plant, total plant weight (g), grain yield/plant (g), 100-grain weight (g), grain number/plant, panicle weight (g) and spikelet fertility (%).

SDS protein and Isozyme Electrophoresis:

Esterase (Est.) and peroxidase (Prox.) isozymes were studied by using polyacrylamide gel electrophoresis according to the method of Davis (1964). Slabs of 6 and 7.5% acrylamide for separation gel and 2.5% acrylamide for stacking gel were used. SDS – Polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) was performed on water soluble protein fractions according to the method of Laemmli (1970) as modified by Studier (1973).

DNA extraction PCR and ISSR analysis:

DNA isolation and purification was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method according to Murray and Thompson (1980). For ISSR analysis, seven primers (844A, 17889A, UBC848, UBC836, UBC842, HB15 and HB12) presented in Table (1) were used.

PCR amplification conditions were as follow: each 25µl PCR reaction solution contained 1.0 µl (50 ng template DNA), 1.0 µl dNTPs (10 mM), 2.5 µl Mg Cl2 (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 2.0 µl of each primer (0.5µM), 0.25 µl Taq polymerase (5u/ µl). The volume was brought up to 25 µl by nuclease-free water. The PCR-ISSR cycling condition involved initial denaturation at 95°C for 2 min followed by 35 cycles of amplification for 35 cycles, template denaturation at 95°C for 1 min (annealing at 48°C for 1 min) and primer extension at 72°C for 2 min, final extension at 72°C for 5 min was given, followed by storage at 4°C. Agarose (1.5%) was used for resolving the PCR products and 1 kb DNA marker as a standard DNA were used in the present study. The run was performed for 1 hour at 50 volt in SDE-PLAS submarine (10cm x 10cm). Bands were detected on UV- trans-illuminator, photographed by Gel documentation system and according to analysis by Phoretix program 1D gel analysis software version 4.01.

Table 1. ISSR primer sequences used in the present study.

No.	Primer	Sequence^{+} 5' → 3'
1	UBC 848	(CA) 8R*G
2	HB12	(CAG) 3GC
3	844A	(CT) 8AC
4	17889A	(CA)6AC
5	UBC 836	$(AG)_{8Y}$*A
6	UBC 842	(GA)8Y*G
7	HB15	(GTG) 3GC

*Y: (C, T) nucleotide bases and R (A, G) nucleotide bases.

RESULTS AND DISCUSSION

Mean performance of the rice genotypes under normal and saline conditions for the studied traits:

The mean performance of the seven rice genotypes for all studied traits under both conditions is shown in Table (2). The desirable highest mean values under the two conditions were observed from Giza177 and Sakha 102 for the heading date. For the plant height, the highest mean values were obtained from the genotype IR29 under normal condition and Sakha102 under saline condition. Concerning chlorophyll content, the highest mean values observed for Giza159 under normal condition and Agami under saline condition. For panicle length, the longest panicles were obtained from Giza159 under both conditions. For number of panicles /plant, Giza178 produced the highest number of panicles/plant under both conditions. Regarding to number of tillers per plant, the highest number was obtained from Giza178 under both conditions. Regarding to plant weight, Agami was the highest under both conditions. The highest grain yield per plant was obtained from Giza178 under both conditions. For 100-grain weight, Giza177 gave the heaviest grains under normal condition. However, Giza159 was superior in this respect under saline condition. For number of spikelets per panicle, the highest mean values were obtained from Agami under normal condition and Giza178 under saline condition.

For panicle weight, the desirable mean for this trait was obtained from Giza178 under both conditions. For spikelet fertility percentage, Giza177 gave the highest mean values for this trait under normal condition, while Giza178 was the best under saline condition.

Table 2. The genotypes mean performance under normal and saline conditions for the studied traits.

No	Genotype	Heading date (days)		Plant height (cm)		Chlorophyll content		Panicle length		Number of panicles per		Number of tillers per plant		Plant weight (g)		Grain yield / plant (g)		100 grain weight		Number of spikelet/		Panicle weight (g)		Spikelet fertility %	
		Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline
1	Giza 159	106	99	98.83	90.93	41.50	35.03	21.43	16.60	14.67	11.0	14.6	10.0	36.83	14.34	9.68	5.07	2.65	2.34	101.7	85.33	2.21	1.84	91.23	85.80
2	Agami	109	112	118.60	95.77	40.99	39.33	19.85	13.50	15.00	10.0	15.6	9.00	43.72	35.45	10.48	7.22	2.35	2.31	111.00	88.67	2.83	1.83	87.53	85.70
3	GZ1368	109	110	100.10	79.37	39.62	31.50	19.90	15.40	18.67	13.0	19.6	12.0	26.95	10.89	9.38	7.01	2.13	1.90	99.00	79.33	2.1	1.34	88.50	80.83
4	Giza 178	105	111	87.20	76.60	39.80	36.40	19.37	15.85	22.00	15.0	21.0	14.0	37.40	22.30	11.41	8.87	2.03	2.00	109.00	97.00	2.86	1.85	91.03	88.87
5	Giza 177	91	97	94.73	81.77	32.67	30.98	19.40	15.25	15.00	8.00	14.6	8.00	27.13	12.63	10.71	5.58	2.65	2.24	88.00	69.33	2.36	1.79	92.33	74.77
6	Sakha 102	91	93	99.20	72.50	34.83	31.51	18.82	13.40	16.00	9.00	14.6	9.00	22.69	7.48	9.78	4.32	2.51	2.20	90.33	70.33	2.26	1.48	78.23	72.78
7	IR29	103	107	86.60	73.20	34.83	32.37	17.19	13.19	20.00	11.0	18.6	10.0	24.10	10.30	8.69	3.45	2.27	1.79	79.00	54.67	1.77	1.24	86.97	78.70

The previous results indicate that Giza 178 has the most desirable traits related to salinity tolerance while IR 29 and Giza 177 have the worst traits related to salinity tolerance. In fact this is quite logic if we consider Giza 178 is the most salt tolerant genotype according to its genetic background (Indica type) and as known, the greatest salt tolerance sources comes from the indica varieties. Cheng *et. al.*, (1996) reported that saline treatment delayed heading MMV and gave the highest yields in both the control and saline water treatments, 5.45 and 2.99 t/ha, respectively, compared with 5.21 and 2.68 t/ha for MLV, 4.63 and 2.75 t/ha for EMV. Aich and Karim (1997) reported that the effect of saline irrigation water on the agronomic characters, such as, plant height was increased with water salinity and increased days to flowering from 129 to 135 days. Zeng and Zeng (2000) studied the effect of salinity and sowing density on panicle length of rice and the relationship of the panicle length at different sowing densities under salinity. They found that salinity effects were highly significant on panicle length. Asch and Woperies (2001) studied salinity tolerance in both the hot dry season and the wet season for surface water drainage at critical growth stages. The results indicated that number of grains/panicle, 1000-grain weight and sterility% were improved regardless season and development stage, but the strongest salinity effected to yield, whereas plants recovered better from stress at seedling stage. Flood water EC<2 ms/ cm^{-1} hardly affected rice yield.

Biochemical and Molecular Analysis

Total Soluble Protein Analysis:

Proteins banding patterns were detected using Coomassi Brilliant blue-R250 for the seven genotypes which have characterized with molecular weight (MW) ranged from 212 – 6.0 KDa (Figures 1 and 2 and Tables 3 and 4). SDS-PAGE showed that all genotypes were characterized by accumulation of a common protein bands of 66 KDa, 38 KDa and 20 KDa that found in salt tolerant genotypes and were absent in the sensitive ones under saline condition.

Claes *et. al.*, (1990) found that stress induced damage to proteins is a likely consequence of salinity and desiccation and provides an explanation for the induction of this protein. Tabaeizada *et. al.*, (1995) found that a 65-KDa protein was accumulated gradually in tomato cv. Starifre leaves during water stress. He-Dy and Yu (1995) found that new protein of Mw 21.8, 22.5, 40.7 and 53.3 KDa were present in the callus and the 53.3 KDa protein was increased by salinity of rice callus under salinity stress. El-Enany (1997) reported that SDS –PAGE analysis extracted proteins revealed that in cultures growth 25 mM NaCl plus praline, extra polypeptides of MW 190, 58 and 26 KDa accumulated as NaCl concentration that was increased in the

medium MW 67 KDa. Hassanein (1999) studied the SDS-PAGE analysis revealed that tomato plants grown under NaCl showed induction 127 and 52 KDa or repression of 260 and 38 KDa in the synthesis of some polypeptides. Dooki et al., (2006) observed that the expression pattern of 13 proteins significantly changed in all panicles in response to stress. We agree with Samaj and Thelen (2007) that abiotic stress included analyses of the effects of water deficit, salt excess, low and high temperatures, high light, and presence of toxic chemicals such as herbicides or heavy metals in the environment. In several instances, proteomics has allowed the identification of novel genes and the characterization of their regulation and function. In other instances, already known function of proteins found to be regulated by stress has allowed the identification of cellular processes involved in the response. Linking proteome variations to physiological and phenotypic changes will make it possible to identify genes and alleles of interest for the selection of plants able to maintain crop yield as high as possible in unfavorable environments.

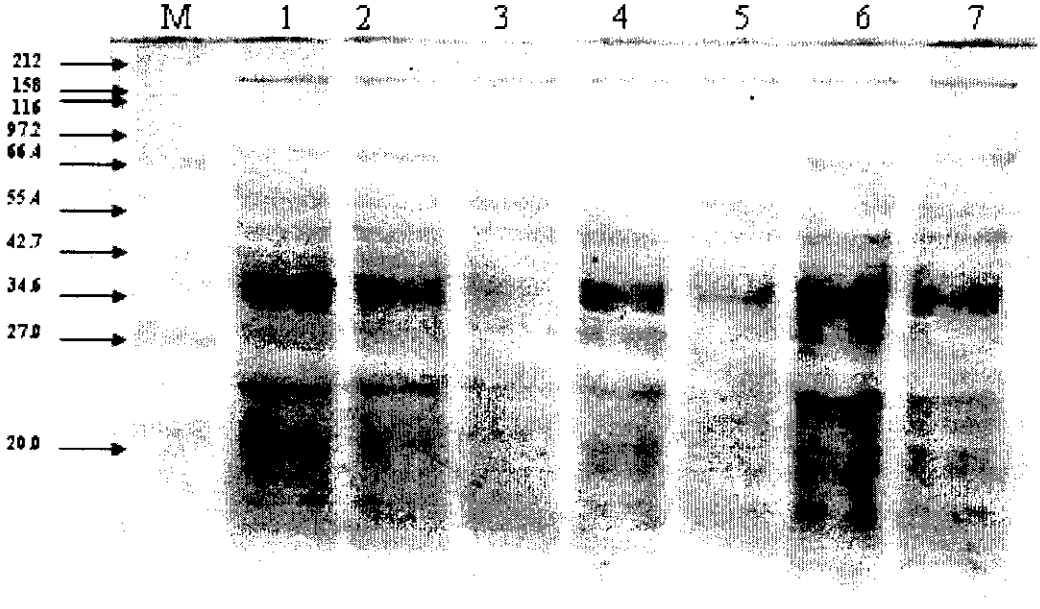


Figure 1. SDS-PAGE of protein banding patterns for seven rice genotypes under normal condition. Lane 1: Giza159, lane 2 : Agami, Lane 3 : GZ1368, Lane 4 : Giza178, Lane 5 :Giza177, Lane 6 : Sakha102 and Lane 7 : IR29.

Table 3. Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from seven rice genotypes under normal condition.

No	MW (KDa)	Lane 1 Giza159	Lane 2 Agami	Lane 3 GZ1368	Lane 4 Giza178	Lane 5 Giza177	Lane 6 Sakha102	Lane 7 IR29
1	212	0	0	0	0	0	0	0
2	158	1	1	1	1	1	1	1
3	116	0	0	0	0	0	0	0
4	97	0	0	0	0	0	0	0
5	66	1	1	1	1	1	1	1
6	56	1	1	0	0	0	0	1
7	53	1	1	1	1	1	1	1
8	43	1	1	1	1	1	1	1
9	39	1	1	1	1	1	1	1
10	37	1	1	1	1	1	1	1
11	35	1	0	1	0	0	0	1
12	32	0	1	0	1	1	1	1
13	27	1	1	1	1	1	1	0
14	25	1	0	0	0	1	0	0
15	23	1	1	1	1	1	1	1
16	21	0	0	0	0	1	1	1
17	20	1	1	1	1	1	0	0
18	18	0	0	0	0	0	1	1
19	16	1	1	1	1	1	1	0
20	15	0	0	0	0	0	0	1

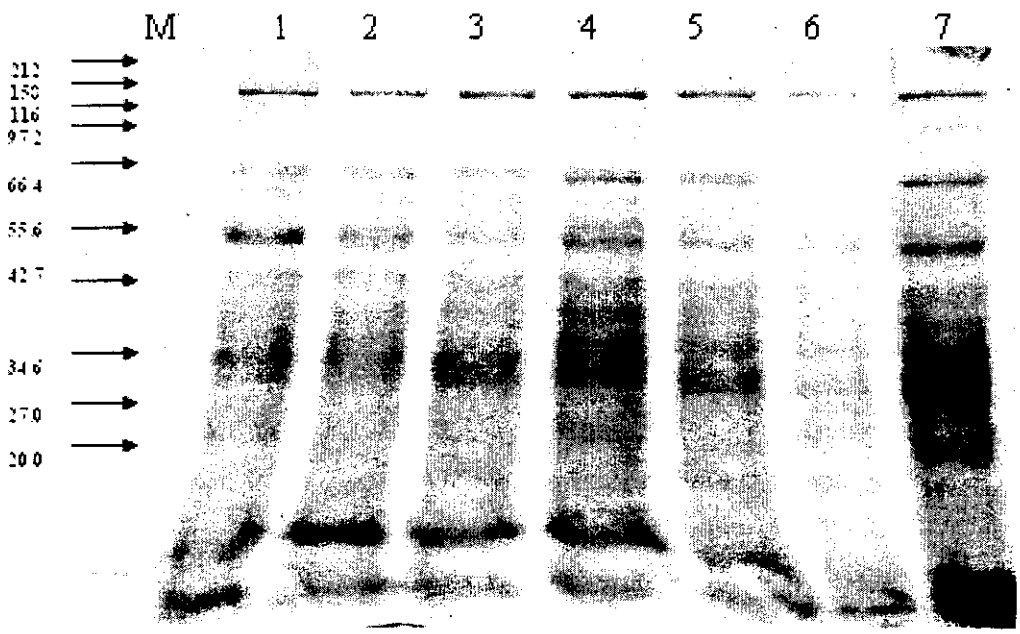


Figure 2. SDS-PAGE of protein banding patterns for seven rice genotype under saline condition. Lane 1: Giza159, lane 2: Agami, Lane 3: GZ1368, Lane 4: Giza178, Lane 5:Giza177, Lane 6: Sakha102 and Lane 7: IR29.

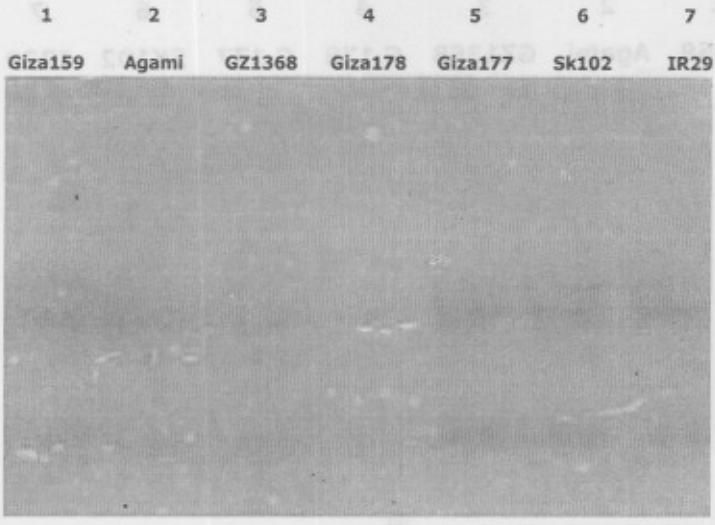
Table 4. Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from seven rice genotypes under saline condition.

No	MW (KDa)	Lane 1 Giza159	Lane 2 Agami	Lane 3 GZ1368	Lane 4 Giza178	Lane 5 Giza177	Lane 6 Sakha102	Lane 7 IR29
1	212	0	0	0	0	0	0	0
2	158	0	0	0	0	0	0	0
3	116	1	1	1	1	1	1	1
4	97	1	0	0	0	0	0	0
5	91	1	1	0	1	1	0	1
6	66	1	1	1	1	1	0	0
7	64	1	1	1	1	1	1	0
8	56	1	1	1	1	1	1	1
9	47	0	0	1	1	1	1	1
10	43	1	1	1	1	1	1	1
11	38	1	1	1	1	0	0	0
12	36	1	1	1	0	1	1	1
13	35	1	1	1	1	1	1	1
14	32	0	0	0	1	1	1	1
15	29	1	1	0	0	1	1	1
16	27	1	1	1	1	0	0	1
17	24	0	0	0	0	1	1	1
18	20	1	1	1	1	1	0	0
19	18	0	0	0	0	0	1	1
20	14	1	1	1	1	0	0	0
21	13	0	0	0	0	1	1	1
22	9	0	0	0	0	0	0	1
23	8	1	1	1	1	0	0	0
24	5	0	0	0	0	1	1	1
25	1	1	0	1	1	0	0	1

Isozyme analysis:

As shown in the esterase zymogram, genotypes, which have salt tolerance exhibited very faint and moderate bands (No.2 and 4) respectively that were presented under saline condition while they were absent in the sensitive genotypes (Figure 3a and b). For peroxidase isozyme, ten bands were exhibited in both conditions for all seven rice genotypes. The results showed that the moderate band (No.10), which was present in the four salt tolerant genotypes and was absent in the sensitive ones. Also, the band (No.4) was present in genotypes Giza159, Agami and Giza 178 (tolerant genotypes) under saline condition on the other hand, band (No.4) was absent in the sensitive genotypes under saline condition (Figure 4a and b).

(A)



(B)

No of bands	1 Giza159	2 Agami	3 Gd368	4 Giza178	5 Giza177	6 Sakha102	7 IR29
1							
2							
3							
4							
5							
6							
7							
8							
9							
Total	8	7	7	7	7	7	7

+++++ :
++++ :
+++ :
++ :
+ :

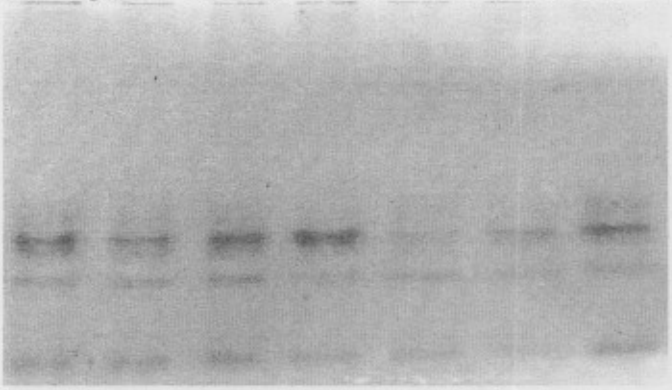
+++++: Very dense, ++++: dense, +++ : moderate, ++: faint, +: very faint

Figure (3a). Isozyme zymogram (A) and diagram (B) of esterase for the seven genotypes under normal condition.

(A)

1 2 3 4 5 6 7

G.159 Agami GZ1368 G.178 G.177 SK102 IR29



(B)

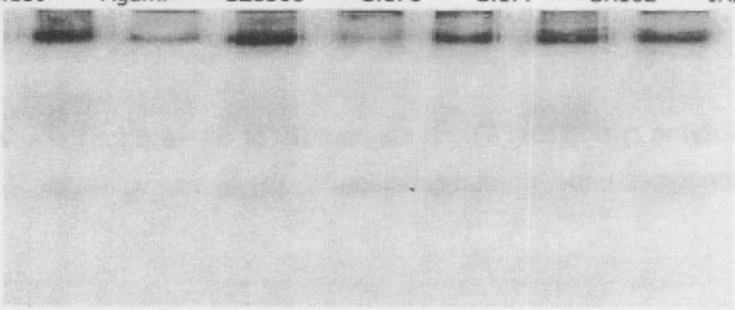
No of bands	1 Giza159	2 Agami	3 Gz1368	4 Giza178	5 Giza177	6 Sakha102	7 IR29
1	—	—		—		—	—
2	—	—	—	—			
3	—	—			—	—	—
4	—	—		—			
5	—	—	—	—	—	—	—
6	—	—	—	—		—	—
7	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—
9	—	—	—	—	—		—
Total	9	9	8	9	6	7	7

Figure (3b). Isozyme zymogram (A) and diagram (B) of esterase for the seven rice genotypes under saline condition.

(A)

1 2 3 4 5 6 7

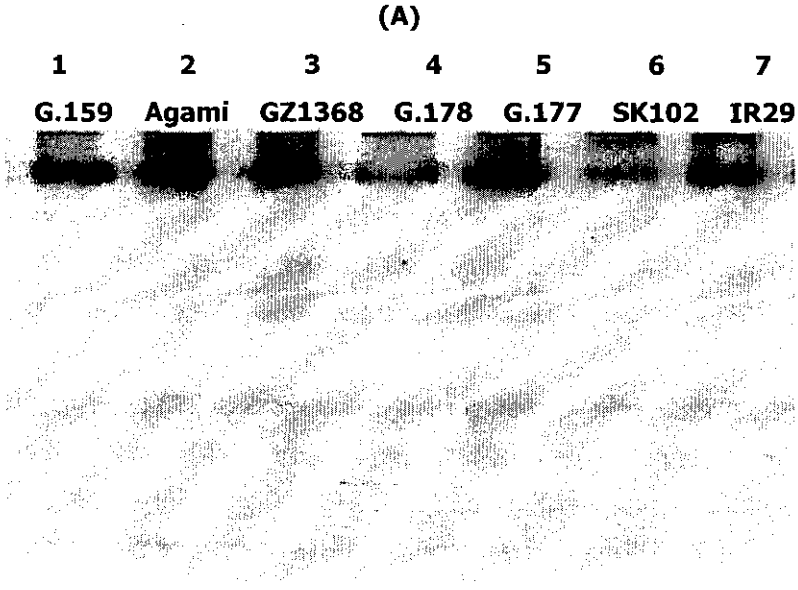
G.159 Agami GZ1368 G.178 G.177 SK102 IR29



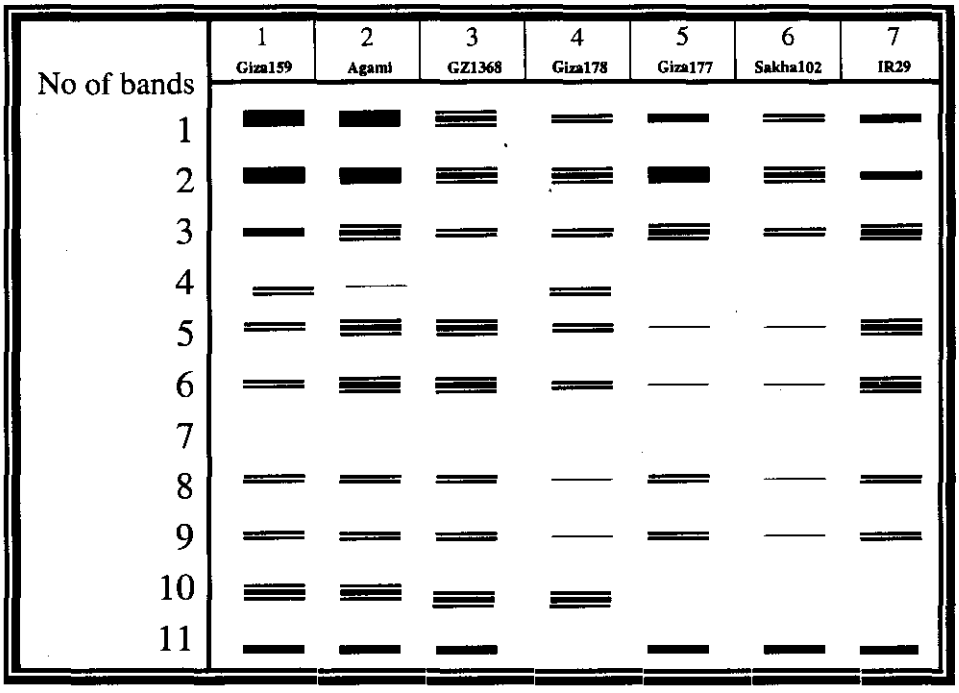
(B)

	1 Giza159	2 Agami	3 GZ1368	4 Giza178	5 Giza177	6 Sakha102	7 IR29
No of bands							
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
Total	**9**	**8**	**9**	**10**	**8**	**9**	**8**

Figure (4a). Isozyme zymogram (A) and diagram (B) of peroxidase for seven rice genotypes under normal soil.



(B)



: Very dense, dense, moderate, faint very faint

Figure (4b): Isozyme zymogram (A) and diagram (B) of peroxidase for seven rice genotypes under saline soil.

DNA markers analysis:

A high level of DNA polymorphism was detected using ISSR technique through the seven genotypes. Primer 1 (UBC 848) showed 13 fragments ranging from 845 to 217 bp. The total number of scorable band was 49 bands. The bands with Mw 400 and 290 bp that were found in the salt tolerant genotypes and were absent in sensitive ones may be considered as markers associated with salt tolerance (Fig.5 and Table 5).

Primer 2 (HB12) showed seven bands ranging from 925 to 350 bp, only one band of them with Mw 925 bp may be considered as positive marker associated with salinity tolerance, and five bands, which have 820, 505,435,390 and 350 respectively, may be considered as negative markers associated with salt tolerance (Fig.6 and Table 6).

Primer 3 showed 10 bands ranging from 740 to 230 bp. The bands with Mw 420, 310, 250 and 230 were common bands. No band can be considered as marker associated with salt tolerance (Fig.7 and Table 7).

Regarding to primer 4, five bands were presented ranging from 650 to 270 bp. The bands with Mw 565, 420 and 345 bp were common bands. The band with Mw 650 bp was found in Giza 159 and Giza 178 and was absent in the remaining genotypes, may be considered as a marker associated with salt tolerance since those two rice genotypes are the most tolerant ones and may be due to those two genotypes have the same mechanisms of salinity tolerance (Fig. 8 and Table 8). Regarding to primer 5 showed five bands ranging from 600 to 380 bp. The band with Mw 600 was present in all tolerant genotypes and was absent in all sensitive ones, this band may be considered as a positive marker associated with salinity tolerance (Fig. 9 and Table 9). Primer 6 showed seven bands ranging from 700 to 120 bp. The bands with Mw 700 and 120 bp were found in all tolerant genotypes while were absent in all sensitive ones. These bands may be considered as positive markers associated with salinity tolerance (Fig. 10 and Table 10). Primer 7 showed nine bands ranging from 600 to 198 bp. The bands with Mw 490 and 430 were present in all tolerant genotypes while were absent in all sensitive ones these bands may be considered as positive markers associated with salinity tolerance (Fig. 11 and Table 11).

Ishii et al., 1996, Doi et al., 2000, Lu *et. al.* (2002), reported that molecular fingerprinting techniques, such as RAPD, was applied to facilitate the assessment. RAPD markers can also provide an efficient assay for polymorphisms, which should allow rapid identification and isolation of chromosome-specific DNA fragments. Wang *et. al.* (1995) analyzed four hundred RAPD primers for screening polymorphism between the genotypes and between two bulks representing fertile and sterile plants, four primers out of them produced polymorphic products. Most of the polymorphic fragments contained repetitive sequences. Using a RAPD analysis, Martin *et. al.* (1997) reported that 16 out of the 93AA genome accessions analyzed had an identity that appeared to be different from that provided by the gene bank. Zhang *et. al.* (1997) identified six RAPD markers as being associated with *Rf-3*. Three of them, OPK05-800, OPU10-1100 and OPW01-350 were mapped on chromosome 1. There have been a number of studies that reported the assessment of genetic or phylogenetic relationships of Oryza species, either including all members in the genus (Aggarwal *et. al.* 1999, Joshi *et. al.* 2000) or only including taxa with the AA-genome. Jingxian *et. al.* (1999) when bulked analysis with RAPDs was conducted, the primer OB19 generated a 1.94 kb RAPD band which differentiated T- and T+ individuals. Wang *et. al.* (1999) studied 16 random amplified polymorphic DNA (RAPD) markers, which were fixed in the F_2 generation of hybrids of Zhongxin 1. These 16 markers were tested using the doubled haploid mapping population of the cross IR 64 x

Azucena, only eight of them were polymorphic for the two genotypes. These eight RAPD markers were located on the IR 64 x Azucena map:

Bair et al., (1999) reported that ISSR fingerprint could be used to differentiate the genotypes belonging to either *Japonica* or *Indica* sub-species of cultivated rice and to dissect finer levels of diversity within each subspecies. A higher percentage of polymorphic bands were produced with the ISSR technique than the AFLP method, based on a similar PCR reaction. Therefore, ISSR amplification proved to be a valuable method for determining genetic variability among rice varieties and for rapidly identifying cultivars. This efficient genetic fingerprinting technique would be useful for characterizing the large numbers of rice accessions held in national and international germplasm centers. Davierwala *et. al.* (2000) used three different marker systems to estimate the genetic diversity of 42 Indian elite rice varieties, and found that RAPD, ISSR and sequence- tagged SSR resulted in mean heterozygosity values of 0.429, 0.675 and 0.882, respectively. Li *et. al.* (2001) reported that the random amplified polymorphic DNA (RAPD) markers OPE 15750 and OPE 15300 were affected by the loss of heterozygosity in rice hybrids AMR x M202 and AMR x L202. Wang *et. al.* (2001) followed random amplified polymorphic DNA (RAPD) markers for the genotypes through the F_3 generation. RAPD markers were uniformly present or absent in all plants within some or all F_2 panicle rows derived from F_1 hybrids involving selected plant (named AMR). In contrast, RAPD markers segregated in the Mendelian manner for dominant markers in panicle rows were derived from control hybrids.

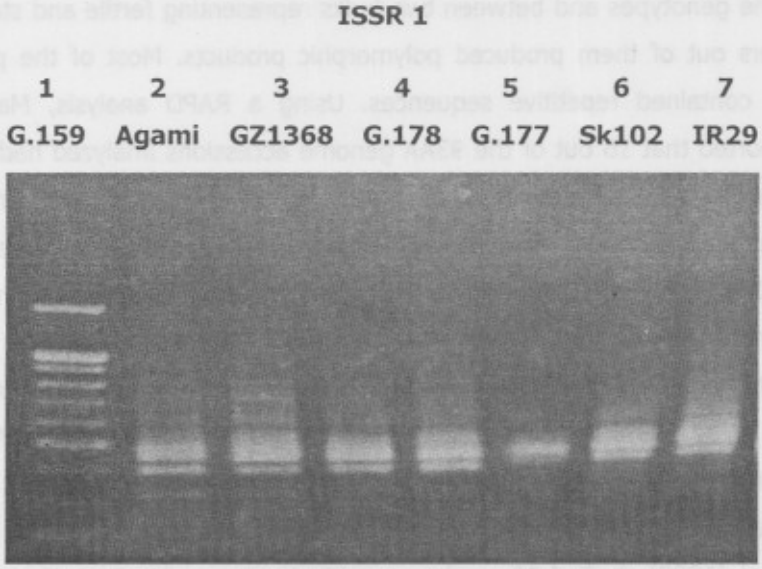


Fig. 5. Genomic amplification using ISSR 1.

Table 5. Presence (1) versus absence (0) of ISSR 1 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
845	0	1	0	0	0	0	0
772	1	0	0	0	0	0	1
685	0	1	1	1	0	0	0
655	1	0	0	0	0	0	1
595	1	1	1	0	1	1	1
510	1	1	1	1	0	1	1
480	1	1	1	1	1	1	1
435	1	1	1	1	1	1	1
400	1	1	1	1	0	0	0
360	0	1	1	1	1	0	0
355	1	0	0	0	0	0	1
290	1	1	1	1	0	0	0
217	0	1	0	0	0	0	0

ISSR 2

1 2 3 4 5 6 7

G.159 Agami GZ1368 G.178 G.177 Sk102 IR29

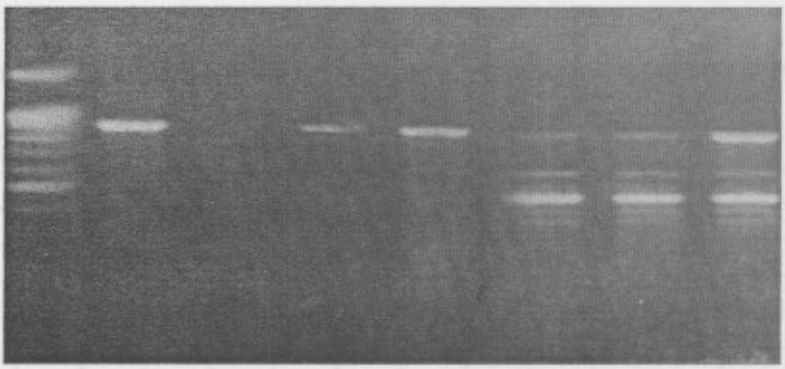


Fig. 6. Genomic amplification using ISSR 2.

Table 6. Presence (1) versus absence (0) of ISSR 2 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
925	1	1	1	1	0	0	0
820	0	0	0	0	1	1	1
590	1	0	0	1	1	1	1
505	0	0	0	0	1	1	1
435	0	0	0	0	1	1	1
390	0	0	0	0	1	1	1
350	0	0	0	0	1	1	1

ISSR 3

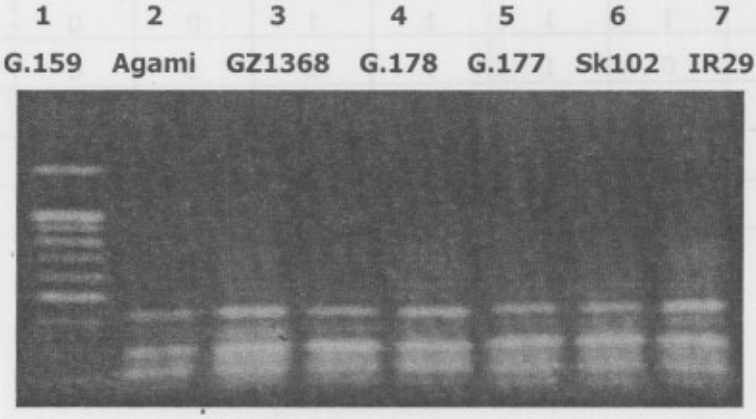


Fig. 7. Genomic amplification using ISSR 3.

Table 7. Presence (1) versus absence (0) of ISSR 3 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
740	0	1	0	1	0	0	1
635	0	1	0	1	0	0	1
550	0	1	0	1	0	0	0
480	1	1	0	1	0	0	0
420	1	1	1	1	1	1	1
380	0	0	0	0	0	1	1
350	0	1	1	1	0	0	0
310	1	1	1	1	1	1	1
250	1	1	1	1	1	1	1
230	1	1	1	1	1	1	1

ISSR 4

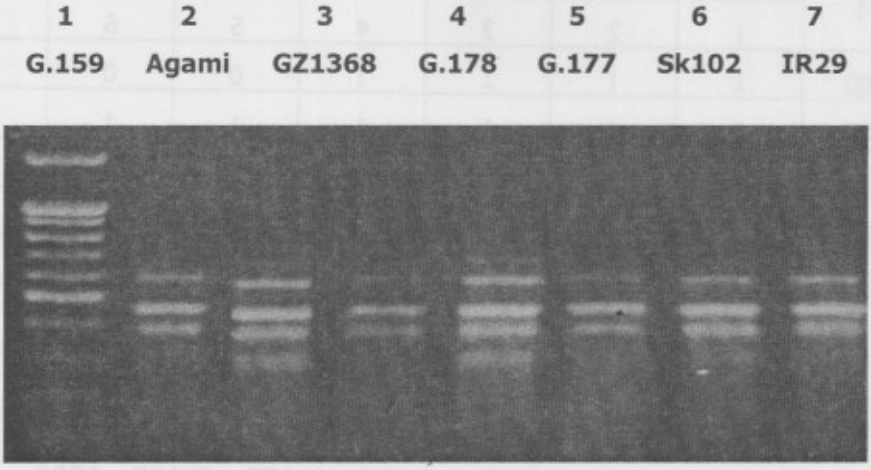


Fig. 8. Genomic amplification using ISSR 4.

Table 8. Presence (1) versus absence (0) of ISSR 4 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
650	0	1	0	1	0	0	0
565	1	1	1	1	1	1	1
420	1	1	1	1	1	1	1
345	1	1	1	1	1	1	1
270	0	1	0	1	0	1	1

ISSR5

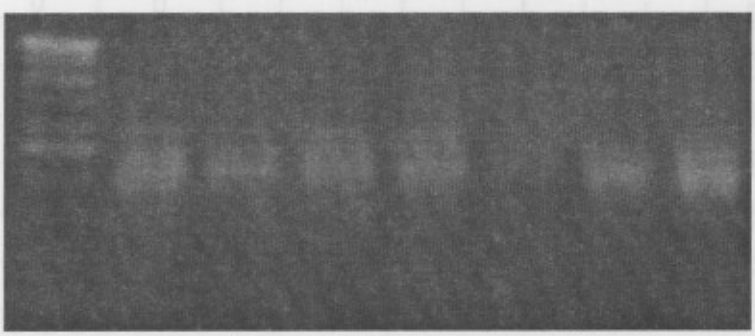


Fig. (9): Genomic amplification using ISSR 5.

Table 9. Presence (1) versus absence (0) of ISSR 5 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
600	1	1	1	1	0	0	0
500	1	1	1	1	1	1	1
450	1	1	1	1	1	1	1
400	1	1	1	1	0	1	1
380	1	0	0	0	0	0	0

ISSR 6

1 2 3 4 5 6 7

G.159 Agami GZ1368 G.178 G.177 Sk102 IR29

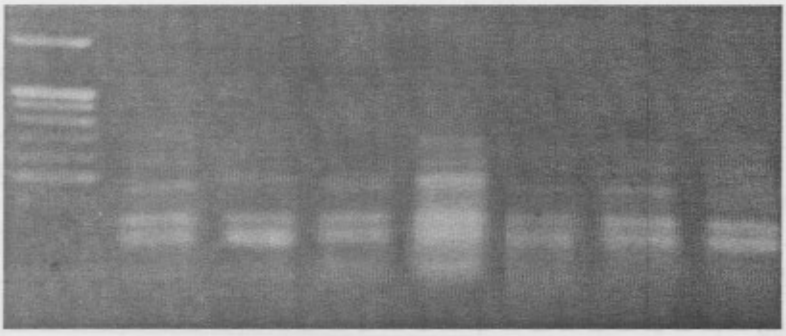


Table 10. Presence (1) versus absence (0) of ISSR 6 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
700	1	1	1	1	0	0	0
570	1	1	0	1	0	1	1
500	0	1	1	1	0	0	0
460	1	1	1	1	1	1	1
300	1	1	1	1	1	1	1
250	1	1	1	1	1	1	1
120	1	1	1	1	0	0	0

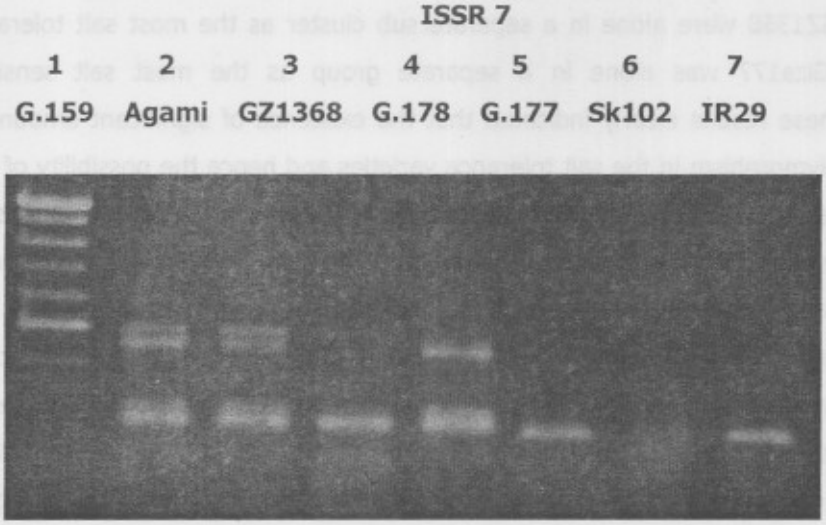


Table 11. Presence (1) versus absence (0) of ISSR 7 primer bands presented in the seven rice genotypes.

M	Tolerant varieties				Sensitive varieties		
	1	2	3	4	5	6	7
600	1	1	0	1	0	0	0
490	1	1	1	1	0	0	0
430	1	1	1	1	0	0	0
350	0	0	0	1	0	0	0
330	1	1	1	1	1	1	1
250	1	1	1	0	0	0	0
230	1	1	1	1	1	1	1
200	0	0	0	1	0	0	0
198	1	1	1	0	0	0	0
188	0	0	0	0	0	1	0

Clustering of the genotypes based on ISSR primers variations:

A dendrogram was constructed based on Numerical Taxonomy System of Multivariate Programs (NTSYS) similarity coefficients, in which the seven rice genotypes were grouped into two major clusters (Fig. 12). Cluster I consisted of the four salinity tolerance rice varieties (Agami, Giza159, Giza178 and GZ1368). Cluster II consisted of the three salt sensitive ones (Giza177, Sakha102 and IR29). Varieties

Agami and GZ1368 were alone in a separate sub cluster as the most salt tolerance genotypes. Giza177 was alone in a separate group as the most salt sensitive genotype. These results clearly indicated that the existence of significant amount of molecular polymorphism in the salt tolerance varieties and hence the possibility of salt tolerant varietal development using such genetic pool under Egyptian conditions. It also emphasizes the importance of molecular markers in germplasm diversity assessment and the strong correspondence between the molecular fingerprints and the genetic behavior of the salt tolerance varieties and their performance. The lack of common band(s) distinguishing salt tolerant varieties from the sensitive ones is basically because of the complexity of the trait and the different mechanisms operating in each variety. This emphasizes the urgent need of molecular mapping for salinity tolerance to detect linked DNA marker(s). Doing so will greatly help in improving selection efficiency for salt tolerance.

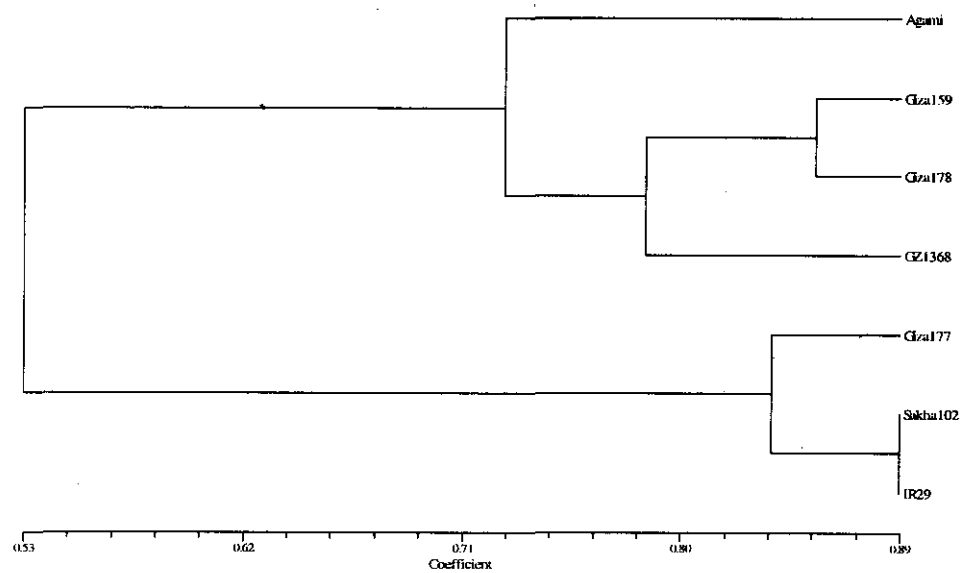


Fig. 12. Dendrogram of the seven rice genotypes based on NTSYS of the seven ISSR primers.

REFERENCES

1. Aggarwal, R.K., D.S. Brar, S. Nandi, N. Huang and G.S. Khush. 1999. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet. 98:1320–1328.
2. Aich, A.C. and F. Karim. 1997. Impact of salinity on growth, yield and salt tolerance limit of HYV rice (Br-16) in coastal saline soil. Bangaladesh water Development Board, New Eskaton (2nd floor), Dhaka 1000, Bangaladesh. J. physiol. Res., 10:1-2, 89-92.
3. Archak S., B. Gaikwad, D. Gautam, V. Rao, M. Swamy and L. Karihaloo. 2003. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (Anacardium occidentale L.) accessions of India. Genome 46: 362-369.
4. Asch, F. and M.C.S. Wopereis. 2001. Responses of field grown irrigated rice cultivars to varying level of flood water salinity in semi-arid environment. Department of Agricultural Science, the Royal Veterinary and Agricultural University, Denmark, Field Crops Res., 70(2):127-137.
5. Bair M. W., O. Panaud and S. R. McCouch. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor. Appl. Genet. 98: 780-792.
6. Bhowmik S. Kumar, T. Soubir, M. I. Mirza, S. Ayesha, S. Sharmin and M. D. Shahidul Haque. 2009. Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. African J. Biotechnology. 8 (23), pp. 6490-6494.
7. Cheng-Jun Wu, Zai-Quan Cheng, Xing-Qi Huang, Shou-Hua Yin,Cheong, Jin, I.L., Bokyeong Kim and H.T. Shin. 1996. Varietal difference of yield and yield components of rice by saline water treatment. National Honam Agricultural Experiment station. RDA, Iksan 570-080, kore Republic, RDA. J. Agric. Sci. Rice, 38(2):12-19.
8. Claes, B., R. Dekeyser, R. Villorroel, Bulckem, B. Bauw and M. Caplan. 1990. Characterization of rice gene showing organ-specific expression in response to salt stress and drought. Pt. Cell,2:19-27.
9. Collard, B.C.Y. and D.J. Mackill. 2007. Marker-assisted selection: An approach for precision plant breeding in the 21st century. Phil. Trans. R. Soc. B. Rev. doi: 10.1098-rstb.2007.2170.
10. Corazza-Munnes M., M. Machado, W. Nunes, M. Cristofani and M. Targon 2002. Comparative assessment of DNA fingerprinting technique (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum*) germplasm. Euphytica 13:135-144.
11. Davierwala, A.P., K.V. Chowdari, S.Kumar , A.P.K. Peddy , P.K. Ranjekar, and V.S. Gupta. 2000. Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. Genetica 108:269–284.

12. Davis, R.J. 1964. Disc electrophoresis 2-method of application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404-427.
13. Doi, K., M.N. Nonomura, A. Yoshimura, N. Iwata, and D.A. Vaughan. 2000. RFLP relationships of A-genome species in the genus Oryza. J Faculty Agric. Kyushu Univ. 45:83–98.
14. Dooki A.D., F.J. Mayer-Posner, H. Askari1, A. Zaiee, G.H. Salekdeh. 2006. Proteomic responses of rice young panicles to salinity. Proteomics 6, 6498–6507.
15. El-Enany, A. 1997. Shoot regeneration and protein synthesis in tomato tissue cultures. Biologia-plantarum, 39 (2):303-308.
16. Hassanein, A. 1999. Alterations in protein and esterase patterns of peanut in response to salinity stress. Biologia-plantarum, 42 (2):241-248.
17. He, Dy. and S.Yu. 1995. Changes in composition of amino acids and proteins of high praline producing variant from rice callus under salt stress. Acta phytophysiologica Sinica, 21(2):123-130.
18. Ishii, T., T. Nakano , H. Maeda and O. Kamijima. 1996. Phylogenetic relationships in A- genome species of rice as revealed by RAPD analysis. Genes Genet Syst. 71:195–201.
19. Joshi, S.P., V.S. Gupta, R.K. Aggarwal, P.K. Ranjekar and D.S. Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple - sequence repeat (ISSR polymorphism in the genus *Oryza*). Theor. Appl .Genet. 100:1311–1320.
20. Jingxian, Z., T.N. Henry and A. Blum. 1999. Genetic analysis of osmotic adjustment in crop plant. J. Exp. Botany, 50:292-302.
21. Khatun, S., C.A. Rizzo and T.J. Flowers. 1995. Genotypic variation in the effect of salinity on fertility in rice. Plant and Soil, 173:239-250.
22. Laemmli, U.K. 1970. Cleavage of structure proteins during assembly of head bacteriophage T4. Nature. 227: 680-685.
23. Li, X.M., R.R.C. Wang, S.R. Larson and N.J. Chatterton. 2001. Development of a STS marker assay for detecting loss of heterozygosity in rice hybrids. Genome. 44: 1, 23 – 26, 12 ref.
24. Lu, B.R., K.L. Zheng, H.R. Qian and J.Y. Zhuang. 2002. Genetic differentiation of wild relatives of rice as referred by the RFLP analysis. Theor. Appl. Genet. 106:101–106.
25. Martin, C., A. Juliano, H.J. Newbury, B.R. Lu , M.T. Jackson and B.V. Ford-Lloyd, 1997. The use of RAPD markers to facilitate the identification of *Oryza* species within germplasm collections. Genet. Res. Crop, 44:175-183.
26. Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4325.
27. Naghia, P.T., J.P.S. Malik, M.P. Pandey and N.K. Singh. 2002. Application of RAPD marker for genetic distance analysis of hybrid rice parental lines. Indian J. Genet. Plant Breed. 62(1): 1 – 4.

28. Palombi M. and C. Damiano. 2002. Comparison between RAPD and SSR molecular markers in detecting genetic variations in kiwifruit (Actinidadeliciosa A. chev.). Plant Cell. Rep. 20 (11): 1061-1063.
29. Saker, M., M. Nagchtigall and T. Kuehne. 2005. A comparative assessment of DNA fingerprinting by RAPD, SSR and AFLP in genetic analysis of some barley genotypes. Egypt. J. Genet. Cytology 34: 81-97.
30. Samaj J. and Thelen J. Jay. 2007. Quantitative Proteomics in Plants: Choices in Abundance. The Plant Cell. 19: 3339–3346.
31. Studier, F.W. 1973. Analysis of bacteriophage T7 early RNAs and protein on slap gels. Mol. Bio.,79:237-248.
32. Tabaeizada, Z., H. chamberland,R. Chen, L. Yu, G. Bellemare and J. Lafontain. 1995. Identification and immunolocalization of a 56 KDa drought induced protein in cultivated tomato Lycopersicon esculentum. Protoplasma, 186(3-4)208-219.
33. Virk S., J. Zhu, H. Newbury, G. Bryan, M. Jackson and B. Ford-Lloyd. 2000. Effectivene of different classes of molecular markers for classifying and revealing variations in rice (*Oryza sativa*) germplasm. Euphytica .112: 275-284.
34. Wang, B., W.W. Xu, J.Z. Wang, W. Wu, H.G. Zheng, Z.Y. Yang, J.D. Ray and H.T. Nguyen. 1995. Tagging and mapping the thermo-sensitive genic and male –sterile gene in rice (Oryza sativa L.) with molecular markers. Theor. Appl. Genet. 91: 6-7.
35. Wang, R.R.C., X.M. Li, S.R. Larson and N.J. Chatterton (1999). Mapping of RAPD markers affected by loss of heterozygosity. Rice Biotechnology Quarterly.40: 16 – 17.
36. Wang, R.R.C., X.M. Li, N.J. Chatterton, S.M. Jain, B.S. Ahloowalia, G.S. Khush and L.H. Zhu. 2001. A proposed mechanism for loss of heterozygosity in rice hybrids. Selected proceedings of the 18th International Congress of Genetics, Beijing, China, 10 – 15 August, Euphytica. 188: (2), 199 –126.
37. Xu, K.-M., M. Zhang, Z.A. Eitzen., S.J. Ghan, S.A. Klein, X. Wu, S. Xie, M. Mranson, A.D. Del Genio, S.F. Iacobellis, M. Khairoutdinov, W. Lin, Ü. Löhmann, D.A. Randall, R.C.J. Somerville, Y.C. Sud, G.K. Walker, A. Wolf, J.J. Yio, and J. Zhang. 2005. Modeling springtime shallow frontal clouds with cloud-resolving and single-column models. J. Geophys. Res., 110.
38. Zeng, L. and L.H. Zeng. 2000. Effects of salinity on grain yield and yield components of rice at different seeding densities. Agron. J. 92(3):418-423.
39. Zhang, G., T.S. Bharaj, Y. Lu, S.S. Virmani and N. Huang. 1997. Mapping of the Rf-3 nuclear fertility – restoring gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. Theor. Appl. Genet. 94(1): 27 – 33.

التعرف على بعض المعلمات الجزيئية و البيوكميائية المرتبطة بتحمل الملوحة في الأرز

ياسر زين العابدين الرفاعى[1] ، منذر عصفور[2] ، عبد السلام عبيد دراز[1]

١ *مركز البحوث و التدريب في الأرز – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية –الجيزة.*

٢ *معهد البحوث و الدراسات البيئية – جامعة المنوفية*

في هذه الدراسة تم استخدام سبعة تراكيب وراثية ذات مدى واسع نسبيا من الاختلافات تبعا لتحملها للملوحة. حيث تم تعريض هذه التراكيب الوراثية لظروف الملوحة و الظروف الطبيعية. و قد تم عمل التحليل بواسطة المعلمات البيوكيميائية لتحديد التعدد المظهري بين التراكيب الوراثية المتحملة للملوحة والحساسة وتحديد الصفات الكمية المرتبطة بتحمل الملوحة للتعرف على الجينات المسئولة عنها. ولقد أوضحت تقنية SDS-PAGE أن الحزم الشائعة للبروتين ٦٦، ٣٨ و ٢٠ كيلو دالتون يمكن اعتبارها من المعلمات المرتبطة بتحمل الملوحة. و لقد أوضحت النتائج في تقنية esterase isozyme أن الحزم الصغيرة و المتوسطة (رقم ٢ و٤) على التوالي يمكن اعتبارها كمعلمات مرتبطة بتحمل الملوحة. و كما اوضح peroxidase isozyme وجود حزمتين متوسطتين (رقم ٤) و (رقم ١٠) و اللتان تعتبران من المعلمات المرتبطة بتحمل الملوحة. كما تم تحديد ثماني حزم موجبة لـــ ISSR و خمسة اخرى سالبة يمكن اعتبارها معلمات جزيئية مرتبطة بتحمل الملوحة.