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Screening of Rice (*Oryza sativa* L.) genotypes with response to salinity by RAPD marker

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Four genotypes of rice (Dandi, CSR-1, IR-36 and GR-3), differing in salt tolerance were grown at 3 and 5 EC (dSm^{-1}) salinity to study the effect of salinity at seedling (15 DAG) stage. The RAPD study indicated total 50.97% polymorphism and the maximum polymorphic *loci* was obtained by OP-D-8. Dandi at 3 EC and 5 EC recorded 100 per cent band sharing whereas the maximum genetic distance was recorded between CSR-1 (control) and GR-3 (5 EC) salinity. The polymorphisms in RAPDs are useful to study gene expression due to salinity in different rice cultivars.

Key words : RAPD, Rice, Salinity.

INTRODUCTION

The complex stress-induced changes in physiology and growth of the plants are often the result of altered patterns of gene expression. Molecular markers based on the DNA sequence are more varied and reliable which can be used to identify and map the genes affecting complex plant traits such as yield as a result of biotic and abiotic stresses.

The more common methods employed for the identification of DNA markers are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP).

RAPD is a fast, easy and efficient method to obtain information on the genetic variation of the plants. It involves the use of "arbitrary" primers (which could be purchased from commercial sources) in a polymerase chain reaction (PCR), resulting in the amplification of several discrete DNA products. Using molecular genetic techniques, the responses of plants to specific abiotic stresses are now better understood. RAPD analysis has been used to determine the interspecific and intraspecific variations in different plant species (Autunes *et al.*, 1997 and Chandra Shekara *et al.*, 2005). RAPD analysis was used to screen for salt tolerance in maize callus lines (Zacchini *et al.*, 1997) and tomato (Foolad and Chen, 1998). The present investigation was aimed to study the genetic distances among different rice genotypes with response to salinity stress.

MATERIALS AND METHODS

Seeds of rice (*Oryza sativa* L.) varieties *viz.*, Dandi, IR-36 and GR-3 were obtained from Main Rice Research Station, Navagam, Gujarat and CSR-1 from the Central Soil Salinity Research Institute, Karnal, Haryana. Seeds were incubated at 45^0C for 48 hrs. in incubator to break the dormancy, soaked in distilled water for overnight and germinated in Petridishes. After sprouting seedlings were inserted and raised on nylon net kept over a Petridish filled with Yoshida nutrient solution. Seedlings were raised at two different salinity levels *i.e.* 3 and 5 EC and compared with control. DNA was extracted from fifteen days old seedlings.

DNA Extraction :

The genomic DNA was extracted from the seedlings of four rice varieties CSR-1, Dandi, IR-36 and GR-3, grown at 3 and 5 EC salinity in Yoshida nutrient solution with control (without salt) by a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Keim *et al.*, 1988) with some modifications. Seedlings (500 mg) from each cultivar were powdered in liquid nitrogen using a pestle and mortar. The resulting powder was transferred to 10 ml centrifuge tube and extracted for 1 hr at 65°C with 5 ml of pre-warmed (65°C for 20 min) extraction buffer. Proteins were extracted with one volume of chloroform: isoamyl alcohol (24:1). The tubes were shaken by slowly inverting slants for 20 minutes. The tubes were centrifuged at 10,000 rpm for 10 min at 4°C and the aqueous layer was taken. Nucleic acids were precipitated with 0.7 volume of isopropanol, washed with 70% ethanol,

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dried at 37°C for 5 min and kept to remove the odor of ethanol. The precipitate was dissolved in 100 ml of TE buffer (1M Tris-HCl pH 8.0, 0.1 mM EDTA) DNA concentration was determined using a UV spectrophotometer at 260 nm and 280 nm (Sambrook *et al*., 1989).

Randomly Amplified Polymorphic DNA (RAPD) :
Oligonucleotide primers were chosen arbitrarily without prior knowledge of the presence of polymorphic markers in the amplification result (Williams *et al.*, 1990). Primers of OP-B, OP-C and OP-D series were initially screened for detecting polymorphism. The following 7 Operon primers were selected to screen response of salt stress in rice varieties. The details of 7 decamer oligonucleotide primers are listed in Table 2. The reaction consisted of 1 ìl DNA, 0.5 ml d NTPs mix, 0.5 ml Taq DNA polymerase, 1.0 ml Primer (10 p moles/ml), 2.5 ml PCR buffer (10 x) with 15 mM $MgCl_2$ and 19.5 ml, Sterile distilled water in 25 ml volume. The DNA amplification was performed in a thermal cycler with initial denaturation 94°C for 5 min followed by 40 cycles of 94°C for 45 sec, 38°C for 30 sec and 72°C for 45 sec. and final extension at 72 °C for 10 min. Amplification products resolved by electrophoresis 1.2% agarose gel containing 5 ml of ethidium bromide (1 mg/ml) in TBE buffer at 80 V (constant) for 60 minutes, visualized under UV illuminator and photographed by Gel documentation system and analyzed by Gene tool.

RESULTS AND DISCUSSION

The primer wise results for marker sequence, scorable bands, total *loci* and polymorphic *loci* were presented in Table 1. The total number of *loci* was 53 out of which 27 (50.97%) were polymorphic. The total polymorphic *loci* ranged from 1-6 and maximum polymorphic *loci* was obtained by primer OP-D 08. The total polymorphic *loci* and per cent polymorphic *loci* with in variety due to salinity was recorded in Table 2. Variety CSR-1 recorded maximum number of polymorphic *loci* (14) with 26.55% polymorphism. Varieties such as IR-36 and GR-3 showed presence of 6 polymorphic *loci* with 11.32% polymorphism. The minimum polymorphic *loci* (4) was observed in Dandi with 7.55 per cent polymorphism. The 2-9 scorable fragments ranged in size from 200-1200 bp were resolved.

Table 2: Polymorphic loci and percent polymorphism with in rice due to salinity.

S. No.	Genotypes	No. of Polymorphic loci	per cent polymorphism
1	Dandi	4	7.55
2	CSR-1	14	26.55
3	IR 36	6	11.32
4	GR 3	6	11.32

Rahman *et al*. (1998) identified DNA markers linked to salt tolerance in wheat genotypes by RAPD markers. Out of the 74 primers, four primers were used (OPA 16, OPM 14, OPR_1 14 and OP_2 10) to produce polymorphic DNA fragments. However, RAPD analysis of DNA from individual plants showed that only a polymorphic DNA fragment of 680 bp amplified by primer OP_2 10 was associated with salt tolerance.

The band sharing (bs) and per cent difference (PD) among rice varieties with salinity were shown in Table 3 and 4. The highest band sharing 100% was observed between Dandi 3 EC and 5 EC salinity; however, genotypes CSR-1 (control) and GR-3 at 5 EC salinity recorded the minimum band sharing (78.48%) with 21.5% per cent differences.

The genetic identity (above diagonal) and genetic distance (below diagonal) was calculated by Nei (1987) and values were presented in Table 5. The maximum genetic distance (0.98) was found between Dandi at 3

Table 1: Primer wise scorable band, total loci and polymorphic loci.

Primer	Sequence	Scorable band	Total loci	Polymorphic loci
OP-B 11	5^1GGACTGGAGT3^1	6-7	7	1
OP-B 13	5^1TTCCCCCGCT3^1	2-5	6	4
OP-D 05	5^1TGAGCGGACA3^1	4-6	6	2
OP-D 06	5^1ACCTGAACGG3^1	6-8	9	5
OP-D 07	5^1TTGGCACGGG3^1	4-6	6	4
OP-D 08	5^1GTGTGCCCCA3^1	6-9	10	6
OP-D 09	5^1CTCTGGAGA3^1	4-7	9	5
Total			53	27

Table 3: Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in rice seedlings with salinity (15 DAG)

G	Dandi			CSR1			IR 36			GR 3		
T	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC
	1	2	3	4	5	6	7	8	9	10	11	12
1	****	0.06	0.08	0.26	0.23	0.14	0.28	0.28	0.31	0.31	0.31	0.19
2	0.94	****	0.02	0.28	0.31	0.21	0.21	0.26	0.23	0.28	0.28	0.16
3	0.93	0.98	****	0.31	0.28	0.19	0.19	0.23	0.21	0.26	0.26	0.14
4	0.77	0.76	0.74	****	0.19	0.28	0.33	0.33	0.31	0.36	0.31	0.39
5	0.79	0.74	0.76	0.83	****	0.12	0.26	0.26	0.33	0.23	0.23	0.21
6	0.87	0.81	0.83	0.76	0.89	****	0.26	0.31	0.33	0.23	0.23	0.12
7	0.76	0.81	0.83	0.72	0.77	0.77	****	0.08	0.06	0.28	0.28	0.21
8	0.76	0.77	0.79	0.72	0.77	0.74	0.93	****	0.10	0.33	0.33	0.31
9	0.74	0.79	0.81	0.74	0.72	0.72	0.94	0.91	****	0.31	0.31	0.28
10	0.74	0.76	0.77	0.70	0.79	0.79	0.76	0.72	0.74	****	0.04	0.10
11	0.74	0.76	0.77	0.74	0.79	0.79	0.76	0.72	0.75	0.96	****	0.10
12	0.83	0.85	0.87	0.68	0.81	0.89	0.81	0.74	0.76	0.91	0.91	****

(C—Control, G—Genotype, T—Treatments)

EC and 5 EC salinity whereas the maximum genetic identity (0.39) was recorded between CSR-1 (control) and GR-3 at 5 EC salinity.

For better understanding the genetic relationships among the varieties with salinity treatments, the dendogram based on Nei's (1978) genetic distance method (UPGMA), modified from Neighbor procedure of PHYLIP version 3.5 was constructed (Fig.1). Variety CSR-1 grown at control was totally out rooted. Rest of the genotypes branched into two groups. Among these IR-36 fall in one group whereas second group composed of Dandi, IR-36 and CSR-1 (only 3 EC and 5 EC salinity), which was further sub-branched. Overall four clusters were observed. Similar genetic distance was recorded

Table 4: Band sharing percentage (BS %) between different genotypes of rice with salinity

G	Dandi			CSR1			IR 36			GR 3		
T	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC
	1	2	3	4	5	6	7	8	9	10	11	12
1	**	97.5	95.1	85.7	85.4	91.6	84.0	84.7	87.5	83.5	82.9	88.6
2		**	100.0	84.0	83.5	87.5	87.2	85.4	85.7	78.9	83.5	86.8
3			**	82.9	85.0	88.9	91.1	86.7	87.2	85.7	85.0	88.3
4				**	90.0	81.9	81.5	75.3	82.5	81.0	82.9	78.5
5					**	91.4	86.1	84.3	82.1	85.7	87.5	88.3
6						**	85.0	81.0	81.0	87.2	84.0	89.7
7							**	93.0	82.5	81.9	83.5	86.8
8								**	93.8	82.5	81.9	80.0
9									**	85.3	82.1	82.7
10										**	93.5	91.9
11											**	93.5
12												**

(C—Control, G—Genotype, T—Treatments)

Table 5: Percent differences (PD %) between different genotypes of rice with salinity.

G	Dandi			CSR 1			IR 36			GR 3		
	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC	C	3 EC	5 EC
T	1	2	3	4	5	6	7	8	9	10	11	12
1	0.0	2.5	4.9	14.3	14.6	8.4	16.0	15.3	12.5	16.5	17.1	11.4
2		0.0	2.0	16.0	16.5	12.5	12.8	14.6	14.3	21.1	16.5	13.2
3			0.0	17.1	15.0	11.1	8.9	13.3	12.8	14.3	15.0	11.7
4				0.0	9.8	18.1	18.5	24.7	17.5	19.0	17.1	21.5
5					0.0	8.6	13.9	15.7	17.9	14.3	12.5	11.7
6						0.0	15.0	19.0	19.0	12.8	16.0	10.3
7							0.0	6.2	17.5	18.1	16.5	13.2
8								0.0	6.2	17.5	18.1	20.0
9									0.0	14.7	17.9	17.3
10										0.0	6.5	8.1
11											0.0	6.5
12												0.0

(C—Control, G—Genotype, T—Treatments)

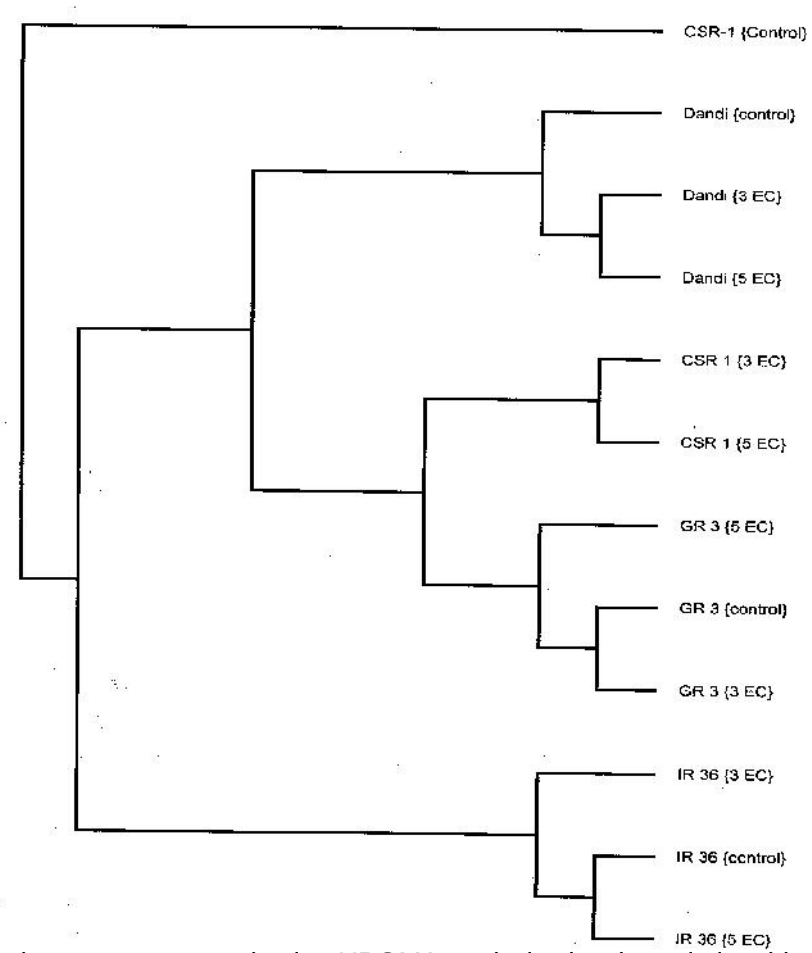

Fig. 1: Dendogram constructed using UPGMA analysis showing relationship among rice genotypes by RAPD analysis.

between Dandi and CSR-1 with 3 and 5 EC salinity. Less genetic distance observed between IR-36 and GR-3.

The study revealed that, the polymorphism in RAPDs is useful to study gene expression due to salinity in different rice cultivars.

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