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Identification of salt tolerant rice genotypes and their genetic diversity analysis using SSR markers

Prabir Dhar[1], M. Ashrafuzzaman[1*], S. N. Begum[2], M. M. Islam[2], M. M. H. Chowdhury[1]

[1]*Department of Genetic Engineering & Biotechnology, School of Life Sciences, Shahjalal University of Science & Technology, Sylhet-3114, Bangladesh*

[2]*Plant Tissue Culture Laboratory, Plant Breeding Division Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh*

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Abstract

Twenty six rice germplasms were used to evaluate salinity tolerance at the seedling stage. Salinity and non-salinized setup were maintained at seedling stage. Phenotyping for salinity screening of the rice genotypes was done using salinized (EC level 12 dS /m) nutrient solution in hydroponic system following IRRI standard protocol. Genotypes were evaluated individually for salinity tolerance on 1-9 scale on the basis of seedling growth parameters following modified SES of IRRI. At the seedling stage, sixteen moderately tolerant and ten susceptible genotypes were identified using Standard Evaluation Score (SES).On the basis of SES score and phenotypic performance, out of 26 rice germplasms two (BINAdhan-8 and AYT SL-3)were selected as compare to other germplasms. For genotypic salt tolerance of 26 rice germplasms, DNA was extracted from leaf samples using CTAB mini-prep method. Then six selected SSR markers viz., RM10701, RM304, RM11757, RM336, RM7075, and RM152 were used for identification of salt tolerant genotypes. The band obtain from reaction with different markers were compared to the selected genotypes (BINAdhan-8 and AYT SL-3). Assessment of genetic diversity is an essential component in germplasm characterization and conservation. In DNA profiling, a total of 60 alleles were detected with an average number of alleles of 10 per locus (range 8 to 12 per locus) and the PIC values ranged from a low of the 0.7459 (RM152) to a high of 0.8908 (RM10701) and averaged 0.857. Positive correlations were found between gene diversity, PIC value and number of allele. The Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram constructed from (Nei's, 1972*)* genetic distance produced two main clusters of 26 rice germplasms. The lowest genetic distance (0.200) was found in CSR-28 vs. CSR90-IR-2 genotype pair indicating that they are genetically much closer among the genotypes. A cluster was consisted with a moderate salt tolerant due to higher similarity, while the mostly susceptible germplasms in the second cluster due to lower genetic distance between germplasms.

*Corresponding Author: M. Ashrafuzzaman ✉ azamanbt@gmail.com

Introduction

Rice, *Oryza sativa* (2n = 24) belonging to the family Graminae and subfamily Oryzoidea, is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals. Rice (*Oryza sativa L.*) is one of the staple for more than 2.7 billion people on a daily basis and is planted on about one-tenth of the earth's arable land (Refaee *et al.,* 2006). Salinity is the most common abiotic problem in rice growing areas of the world. Salinity causes unfavorable environment and hydrological situation that restrict the normal crop production throughout the year (Haque, 2006). Salinity causes unfavorable environment and hydrological situation that restrict the normal crop production throughout the year (Haque, 2006). In Bangladesh, rice production may fall by 10 % and wheat by 30 % by 2050 (IPCC, 2007). (Krishnamurthy *et al*., 2009) stated that increasing soil salinity reduces crop yield worldwide, with rice being particularly affected. Microsatellites or simple sequence repeats (SSRs) are simple sequence of tandem repeats which can presently be a short motif of di-nucleotides, or tri-nucleotides, or tetra nucleotides repeated and contains in 1-6 base pairs (bp) in length (Li *et al*., 2004). Molecular marker technology provides a powerful tool in the assessment of genetic relationships within and among species, in which differences among accessions can be revealed at the DNA level (Chakravarthi and Naravaneni, 2006). The applications of microsatellite markers in rice research include studies on genetic diversity of Yunnan rice germplasm by (Tu *et al*., 2007), genetic diversity analysis of traditional and improved Indonesian rice germplasms by (Thomson *et al*., 2007), and other general rice genetic diversity studies by (Ni *et al*., 2002, Ravi *et al*., 2003, Chakravarthi and Naravaneni, 2006). The molecular characterization information as well as genetic diversity analysis could be helpful to the breeders for further planning of rice breeding program to improve grain quality, yield quality and specially for the stress such as salinity, cold, flood etc. tolerant genotype development. Considering the above facts the present study was carried out with the following objectives screening of rice germplasms under salinized condition for the identification of salt tolerant genotypes and to find out genetic diversity among the genotypes for the use of breeding program in future.

Materials and methods

Plant materials

The experiment was carried out in the glasshouse and biotechnology laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Bangladesh. Twenty six rice germplasms with diversified genetic background were used in this study.

This germplasms were obtained from the Bangladesh Institute of Nuclear Agriculture (BINA). The list of used germplasms is shown in following.

SL. No	Variety	Source	SL. No	Variety	Source
1.	BINA dhan-5	BINA	14.	AYT54	BINA
2.	BINA dhan-7	BINA	15.	AYT SL-23	BINA
3.	BINA dhan-8	BINA	16.	AYT SL -1	BINA
4.	BR-47	BINA	17.	AYT SL-3	BINA
5.	BR-47 200G'	BINA	18.	AYT SL-7	BINA
6.	BR-47 250G'	BINA	19.	AYT SL-32	BINA
7.	BR-47 300G'	BINA	20.	AYT SL-41	BINA
8.	BR-47 350G'	BINA	21.	AYT SL-57	BINA
9.	BRRI-28	BINA	22.	CSR-28	BINA
10.	PBRC-30	BINA	23	CSR 90- IR-2	BINA
11.	PBRC-37	BINA	24.	IR 73571-3B-14-2	BINA
12.	FL-378	BINA	25.	IR74095-AC 45	BINA
13.	FL-478	BINA	26.	IE71829-3R82-1-1	BINA

Phenotypic study of salinity tolerance at seedling stage

The genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (Gregorio *et al*., 1997). Salinized and non-salinized setups with 3 replications were maintained. The evaluation was done using (Yoshida *et al*., 1976) nutrient solution at the glasshouse. The nutrient solution was salinized by adding crude salt to obtain desired EC (12 dS/m). The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI, 1997). Visual rating of salinity tolerance was done according to table 1.This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 13 days and 22 days after salinization. For phenotypic observation plant height and root length and total dry matter was recorded at salinized and non-salinized conditions.

Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Source: (Gregorio *et al*., 1997).

Data Collection

The data were recorded from the screening at seedling in both normal and salinized conditions following Standard Evaluation System of IRRI (Gregorio *et al*., 1997). Plant height was measured from the soil surface to top of the rice leaf at seedling stage. Leaves, shoots and roots of individual rice plant was kept into separate packet and oven dried in 70^0 C for a week and weighted. Percent of reduction of total dry matter, percent reduction of shoot length and root length were calculated as follows:

Total Dry Matter (TDM) = dry weight of leaves + dry matter of roots + dry matter of shoots.

Percent (%) reduction of total dry matter (RTDM) = {(TDM at control condition- TDM at saline condition) / TDM at saline condition} ×100.

Percent (%) reduction of shoot length (RSL) = {(shoot length at control condition-shoot length at saline condition)/ shoot length at saline condition}×100.

Percent (%) reduction of root length (RRL) = {(root length at control condition-root length at saline condition)/ root length at saline condition}×100.

Data analysis

MSTATC software was used to perform the phenotypic data at normal and saline condition.

Genotyping of salinity tolerance

DNA samples were extracted from young leaves of 25-days old seedlings and using modified CTAB mini preparation method. In this study twenty primers namely RM556, RMOSR34, RMOSR17, RMOSR32, RMOSR30, RM336, RM21, RM152, RM21, RM51, RM10701, RM10772, RM11757, RM6613, RM7075, RM304, RM234, RM13, RM6613 and RM6386 were used for parental survey. Among them six polymorphic SSR markers viz., RM10701, RM304, RM336, RM7075, RM152 and RM11757 were selected to evaluate 26 rice germplasms for salt tolerance. For microsatellite assay PCR amplification carried out with 15.0 µl containing 0.5 µl of 10 X buffer, 0.75 µl of dNTPs, 1.0 µl of primer forward and 1.0 µl of primer reverse, 0.5 µl of taq polymerase, 8.25 µl of sterilized ddH_2O and 2 µl of each template DNA. The PCR initially denatured at 94^oC for 5 min followed by 34 cycles of 94^oC for 1 min., Primer Annealing at 55^oC for 1 min and 2 min for Primer Extension at 72^oC, finally 7 min incubation at 72^oC.For checking amplification, the PCR products were electrophoreticaly resolved on 1.5% agarose gel in 0.5X TBE. The gel was soaked in ethidium bromide (10 mg/ml) solution for 15-20 min. The gels were viewed by the GEL Doc. Banding pattern of germplasms were scored with BINAdhan-8 and susceptible line AYT SL -3. Allele frequencies were calculated directly from the observed genotypes. Polymorphisms information content (PIC) value of a marker was calculated according to a simplified version after (Andersons *et al*., 1993). PIC was calculated using allele number for each primer. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was drawn by using the software TREEVIEW (Page 1996).

Results & Discussion

Screening of germplasms for salt tolerance at seedling stage

Twenty six rice germplasms were used for screening salinity tolerance. After two or three days of salinization, salt stress symptoms were started. Several salt injuries observed in the salinized conditions which were: leaves became yellowish, drying of leaves, shoot growth reduction, root growth reduction, stem became thin and week, stunted growth of seedlings and seedling drying

occurred. These symptoms were also observed by several researchers (Bhuiyan, 2005, Islam, 2004, Niones, 2004 and Bonilla et al., 2002). The seedlings in the non-salinized condition showed normal growth over the salinized condition. The salinized and non-salinized setups of 26 rice genotypes are shown in Fig. 1.

In the salinized setup, 26 germplasms showed wide variation in phenotypes under salt stress. Among the 26 germplasms, 16 were moderately salt tolerant, 10 were susceptible (Table 3).

Table 2. Sequences of microsatellite markers used for this study.

Primer name	Expected PCR product size (bp)	Chrom. position	Repeat Motif		Primer sequence	Annealing Temp.(°c)
RM10701	69	1	(AG)10	For	GAGACACGGCACAATATACAACG	55
				Rev	TTCTATCTCCGACCTCTTCTCAAGG	
RM304	160	10	(GT)2(AT)10(GT)33	For	TCAAACCGGCACATATAAGAC	55
				Rev	GATAGGGAGCTGAAGGAGATG	
RM11757	598	1	(TTG)48	For	GCTTGTTGCCTGTGAACAGTAGC	55
				Rev	TGTCAGCATGCAACATCAATCC	
RM336	154	7	(CTT)18	For	CTTACAGAGAAACGGCATCG	55
				Rev	GCTGGTTTGTTTCAGGTTCG	
RM7075	155	1	(ACAT)13	For	TATGGACTGGAGCAAACCTC	50
				Rev	GGCACAGCACCAATGTCTC	
RM152	151	8	(GGC)10	For	CCAAGGGAAAGATGCGACAATTG	55
				Rev	GTGGACGCTTTATATTATGGG	

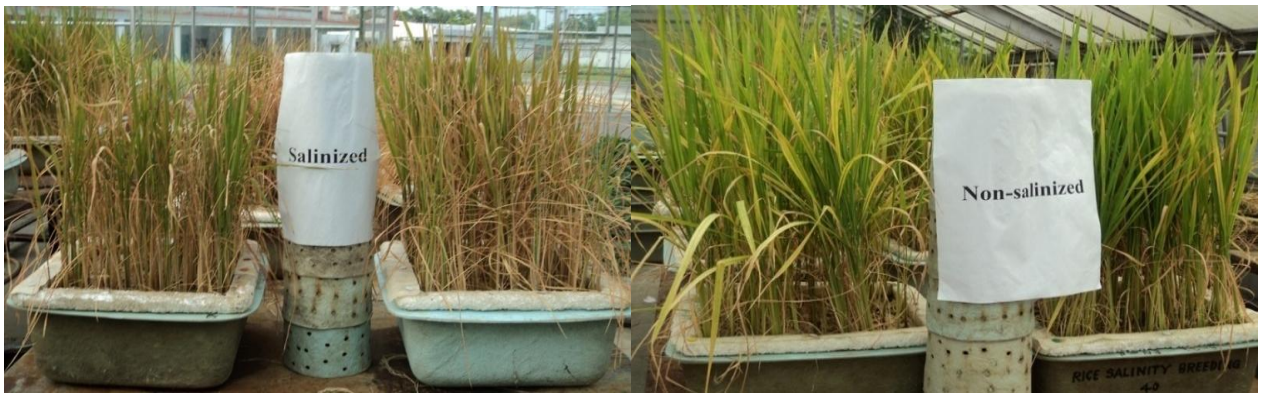

Fig. 1. Setups of salinized and non-salinized conditions of 26 rice germplasms at the Seedling stage using hydroponic system with EC level 12 dS m^{-1} at the glasshouse of BINA.

Salinity affected the shoot length, total dry matter and root length. Under salinity stress there were lower reduction in shoot length of varieties BR-47 (5.07%), BR-47 200GY (10.32%), FL-478(18.19%), IE71829-3R82 (18.36%), PBRC-37(19.13%) (Table 4).Highest reduction (35.70%) in shoot length was observed by the variety BINA dhan-5(35.70%) followed by AYT SL-3(35.45%), BINA dhan-7 (34.27%), AYT SL -1(32.95%), CSR 90 IR-2(31.91%).This results indicate that plant of height decreased under salinity stress.

Salinity decreased root length of tested genotypes. At seedling stage, some genotypes showed higher root length reduction viz. AYT SL -1(43.08%), AYT SL-3 (37.75), IR74095-AC (37.37%), AYT SL-23 (37.04%) and IR 73571-3B (36.74%). On the other hand some genotypes showed lower percentage of reduction in root

length viz., BINA dhan-8 (2.25), CSR-28 (3.16%), BR-47 (5.19%), BR-47 200GY (6.43%) and IE71829-3R (9.5%).Salt stress decreased total dry matter of rice seedlings. Under salinity stress some genotypes showed higher reduction of total dry matter these include AYT SL-3(51.39%), AYT SL-32 (49.32%), AYT SL-23 (41.9%), AYT-54 (40.54%), BINA dhan-5(38.89%).Some genotypes showing lower reduction were BR-47 200GY(1.34%), PBRC-30(1.82%), BR-47control (1.89%), BINA dhan-8(2.23%), BR-47 250GY (3.20%) (Table 4).

Table 3. Performance of the germplasms under salinized condition (EC 12 dS m^{-1}) at the seedling stage.

Sl. No.	Name of germplasm	SES scoring	Tolerance	Sl. No.	Name of germplasm	SES scoring	Tolerance
1.	BINA dhan-5	7	S	14.	AYT54	7	S
2.	BINA dhan-7	7	S	15.	AYT SL-23	7	S
3.	BINA dhan-8	5	MT	16.	AYT SL -1	8	S
4.	BR-47	5	MT	17.	AYT SL-3	8	S
5.	BR-47 200GY	5	MT	18.	AYT SL-7	7	S
6.	BR-47 250GY	5	MT	19.	AYT SL-32	5	MT
7.	BR-47 300GY	5	MT	20.	AYT SL-41	5	MT
8.	BR-47 300GY	5	MT	21.	AYT SL-57	7	S
9.	BRRI-28	7	S	22.	CSR-28	5	MT
10.	PBRC-30	7	S	23	CSR 90 IR-2	5	MT
11.	PBRC-37	5	MT	24.	IR 73571-3B-14-2	5	MT
12.	FL-378	5	MT	25.	IR74095-AC 45	5	MT
13.	FL-478	5	MT	26.	IE71829-3R82-1-1	5	MT

Table 4. Performance of Shoot length, Root length and Total dry matter of 26 rice genotypes at seedling stage (35 Days).

Genotypes	Shoot length(cm)			Root length(cm)			Total dry matter(gm)		
	Non-salinized	salinized	Reduction (%)	Non-salinized	salinized	Reduction (%)	Non-salinized	salinized	Reduction (%)
BINA dhan-5	52.73	33.91	35.70	21.56	17.16	20.41	0.72	0.44	38.89
BINA dhan-7	50.93	33.48	34.27	19.56	17.32	11.46	0.48	0.36	25
BINA dhan-8	53.10	41.20	22.42	17.4	17.01	2.25	0.58	0.57	2.23
BR-47	48.76	46.29	5.07	17.16	16.27	5.19	0.53	0.59	1.89
BR-47200GY	49.53	44.42	10.32	16.96	15.87	6.43	0.60	0.60	1.34
BR-47 250GY	54.73	43.98	19.65	19.86	16.89	17.48	0.65	0.72	3.20
BR-47 300GY	56.20	44.19	21.38	19.6	16.78	14.39	0.58	0.53	9.56
BR47350GY	59.70	47.31	20.76	18.8	16.10	14.37	0.73	0.67	8.22
BRRI-28	53.56	40.63	23.86	33.5	26.31	21.70	0.63	0.52	17.31
PBRC-30	49.73	39.07	21.24	3.83	26.84	20.67	0.55	0.54	1.82
PBRC-37	55	44.48	19.13	27.73	19.47	29.79	0.82	0.65	20.74
FL-378	49.10	39.66	19.23	24	20.02	16.59	0.61	0.58	4.92
FL-478	58.03	47.48	18.19	18.66	15.30	18.01	0.97	0.70	28.28
AYT-54	52.80	38.12	27.81	29.56	18.94	35.93	0.79	0.47	25.83
AYT SL-23	50.83	36.41	28.37	24.33	15.32	37.04	0.74	0.43	41.90
AYT SL -1	56.30	37.75	32.95	29.3	16.68	43.08	0.58	0.48	17.25
AYT SL-3	69.43	44.82	35.45	31.13	19.39	37.75	0.72	0.35	51.39
AYT SL-7	64.4	50.11	22.19	34.76	23.79	31.56	0.63	0.55	12.70
AYT SL-32	41.80	33.36	20.20	28.86	19.53	32.33	0.69	0.35	49.28
AYT SL-41	55.33	42.56	23.08	31.5	21.38	32.13	0.99	0.75	24.70
AYT SL-57	60.93	42.99	29.45	32	20.98	34.44	1.15	0.75	34.88
CSR-28	55	42.18	23.31	18.71	18.10	3.16	0.81	0.71	12.35
CSR 90 IR-2	63.40	43.17	31.91	27.6	23.03	16.56	0.83	0.72	13.88
IR 73571-3B-14-2	56.36	42.93	23.83	30.26	19.08	36.74	0.70	0.58	17.15
IR74095-AC 45	60.13	42.40	29.49	26.6	16.66	37.37	0.92	0.66	28.28
IE71829-3R82-1-1	41.50	33.93	18.36	24.2	21.72	9.50	0.42	0.35	16.67
$LSD_{(0.05)}$	0.778**	4.648**		0.843**	4.485**		0.015**	0.284*	

Evaluation of 26 rice germplasms using SSR markers

The seedlings were evaluated according to salt tolerance using 6 SSR markers. The bands obtained from reactions with different markers were compared to those of moderate tolerant variety BINAdhan-8 and susceptible line AYT SL -3.

When 26 samples were amplified with RM10701 then AYT SL-1, AYT SL-3, AYT SL-7, AYT SL-41 and AYT SL-57 were identified as susceptible and the rest 20 genotypes were identified as moderately tolerant. In the reaction with RM11757 nine genotypes namely BINA dhan-7. PBRC-37, AYT54, AYT SL-1, AYT SL-7, AYT SL-41, AYT SL-57, IR74095-AC, and IE71829-3R were identified as susceptible. In the same reaction the rest ten genotypes were identified as moderately tolerant (BINA dhan-8, BR-47 200GY, BR-47 250GY, BR-47 350GY, BRRI-28, PBRC-30, FL-378, AYT SL-32, CSR-28, and CSR 90 IR-2) (Fig. 2).

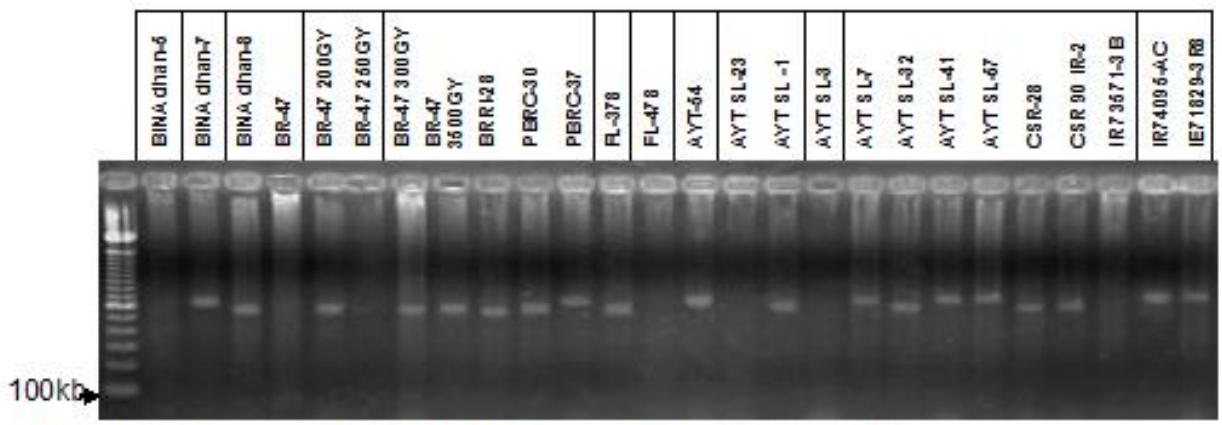

Fig.2: Microsatellite profile of 26 rice genotypes at loci RM11757.

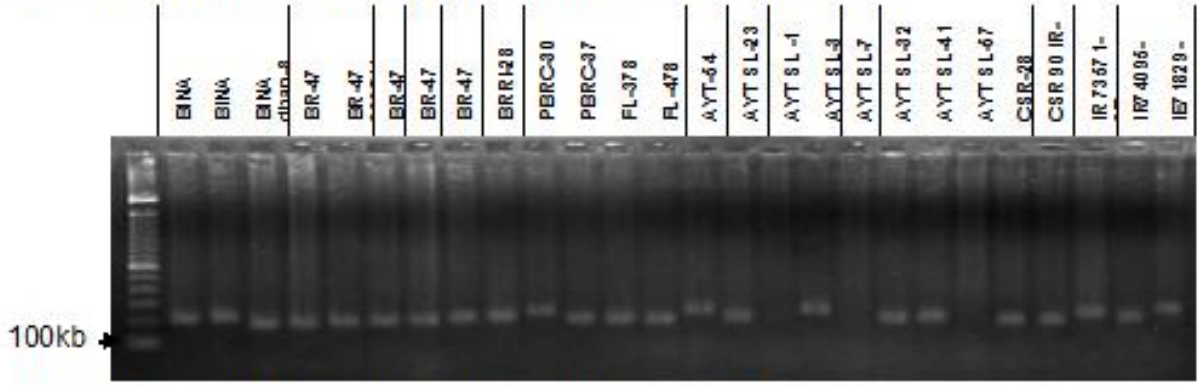

Fig.3: Microsatellite profile of 26 rice genotypes at loci RM336.

In the reaction with RM336 seven genotypes namely BINA dhan-5, BINA dhan-7, PBRC-30, AYT SL-3, AYT54, IR 73571-3B and IE71829-3R were identified as susceptible and the rest genotypes namely BINA dhan-8, BR-47, BR-47 200GY, BR-47 250GY, BR-47 300GY, BR-47 350GY, BRRI-28, PBRC-37,FL-378,FL-478, AYT SL-23, AYT SL-32, AYT SL-41, IR74095-AC , CSR-28, and CSR 90-IR-2 were identified as moderately tolerant (Fig. 3).

Number of alleles per locus

Using 6 SSR markers, a total of 60 alleles (Jain et al., 2004) were detected among the 26 rice germplasms. The average number of allele per locus was 10, with a range of 8 (RM152) to as many as 12 (RM7075 & RM10701) (Table 6).

Table 5. Genotyping scoring of bands of 26 rice germplasms compared with the banding pattern of their selected rice germplasms using 6 SSR markers.

Name of rice germplasm	Markers RM10701	RM304	RM11757	RM336	RM7075	RM152
BINA dhan-5	MT	MT		S	MT	MT
BINA dhan-7	MT	MT	S	S	MT	MT
BINA dhan-8	MT	MT	MT	MT	MT	MT
BR-47	MT	MT		MT	MT	MT
BR-47 200GY	MT	MT	MT	MT	MT	MT
BR-47 250GY	MT	MT	MT	MT	MT	MT
BR-47 300GY	MT	MT		MT	MT	MT
BR-47 350GY	S	MT	MT	MT	MT	MT
BRRI-28	MT	MT	MT	MT	MT	MT
PBRC-30	MT	MT	MT	S	MT	MT
PBRC-37	MT	MT	S	MT	MT	MT
FL-378	MT		MT	MT	MT	MT
FL-478	MT	MT		MT	MT	MT
AYT54	MT	MT	S	S	S	Het
AYT SL-23	MT	MT		MT	S	S
AYT SL-1	S	S	S		S	S
AYT SL-3	S	S		S	S	S
AYT SL-7	S	S	S		S	S
AYT SL-32	MT	S	MT	MT	S	MT
AYT SL-41	S	S	S	MT	S	
AYT SL-57	S	S	S		S	S
CSR-28	MT	Het	MT	MT		MT
CSR 90 IR-2	MT	MT	MT	MT	MT	MT
IR 73571-3B	MT	Het		S	MT	MT
IR74095-AC	MT	MT	S	MT	MT	MT
IE71829-3R	MT	MT	S	S	MT	MT

Table 6. Data on repeat motif, number of alleles, number of rare alleles and gene diversity (GD) found among 26 rice genotypes for 6 microsatellites (SSR).

Locus	Repeat Motif*	No. of alleles	*Rare alleles	Allele Size ranges(bp)	Difference (bp)	Gene Diversity
RM10701	(AG)10	12	3	126-191	65	0.8994
RM304	(GT)2(AT)10(GT)33	9	3	110-215	105	0.8544
RM11757	(TTG)48	9		541-717	176	0.8421
RM336	(CTT)18	10	3	169-247	78	0.8733
RM7075	(ACAT)13	12	4	122-170	52	0.8832
RM152	(GGC)10	8	4	128-176	48	0.7743
Mean		10	3.4			0.8544

Table 7. Data on sample size, number of observation, major alleles (size and frequencies), and polymorphism information content (PIC) found among 26 rice genotypes for 6 SSR markers.

Locus	Sample size	No. of observation	Major allele Size(bp)	Frequency (%)	PIC	Mean PIC
RM10701	26	26	144&149	15.38	0.8908	
RM304	26	25	200&207	20	0.8375	
RM11757	26	19	557	26.32	0.8243	
RM336	26	23	201	21.74	0.8606	0.8387
RM7075	26	25	122	24	0.8733	
RM152	26	24	141	37.5	0.7459	
Mean	26	23.6667	227.5 & 229.5	24.15	0.8387	

*Major allele is defined as the allele with the highest frequency.

*Motif of the SSR and number of repeats as previously published (http://www.gramene.org). **Rare alleles are defined as alleles with a frequency less than 5%.

Rare alleles

An allele observed in less than 5% of the 26 accessions was considered to be rare allele (Jayamani *et al.*, 2007). Rare alleles were observed at all of the SSR loci in one or more of the 26 accessions with an average of 3.4 rare alleles per locus and a total of 17 across all the loci (Table 6). Marker RM7075 detected the greatest number of alleles (12), which also detected higher number of rare alleles (4) and this result agree with (Jain *et al.*, 2004) who also found same output. Rare alleles are highly informative in fingerprinting of the varieties.

Table 8. Summary of (Nei's, 1972) genetic distance values for 26 rice germplasms.

OTU	AYT 54	AYT SL - 1	AYT SL-23	AYT SL-3	AYT SL-32	AYT SL-41	AYT SL-57	AYT SL-7	BINA dhan -5	BINA dhan -7	BINA dhan -8	BR-47	BR-47 200G	BR-47 250G	BR-47 300G	BR-47 350G	BRRI -28	CSR-28	CSR90 IR-2	FL-378	FL-478	IE1829	IR73571	IR74095	PBRC-30	PBRC-37
AYT 54	0.000																									
AYT SL -1	0.800	0.000																								
AYT SL-23	0.800	0.750	0.000																							
AYT SL-3	0.600	0.750	1.000	0.000																						
AYT SL-32	1.000	0.750	1.000	0.750	0.000																					
AYT SL-41	1.000	1.000	0.800	1.000	0.800	0.000																				
AYT SL-57	1.000	1.000	1.000	1.000	0.750	0.600	0.000																			
AYT SL-7	1.000	0.800	1.000	0.500	0.750	1.000	0.800	0.000																		
BINAdhan-5	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	0.000																	
BINAdhan-7	0.833	1.000	1.000	1.000	0.800	0.833	1.000	1.000	0.500	0.000																
BINAdhan-8	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	0.750	0.333	0.000															
BR-47	1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	0.800	0.600	0.000														
BR-47 200G	0.833	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	0.800	0.000													
BR-47 250G	1.000	1.000	1.000	1.000	1.000	1.000	0.750	0.750	1.000	1.000	1.000	1.000	0.800	0.000												
BR-47 300G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	0.800	0.666	1.000	0.000											
BR-47 350G	1.000	1.000	1.000	1.000	0.600	1.000	0.800	0.800	1.000	1.000	1.000	0.800	0.833	0.600	0.833	0.000										
BRRI-28	0.833	1.000	0.800	1.000	1.000	0.833	1.000	1.000	1.000	0.833	0.833	0.800	1.000	1.000	0.666	0.833	0.000									
CSR-28	1.000	1.000	1.000	1.000	0.750	0.600	1.000	1.000	1.000	0.800	0.800	0.750	1.000	1.000	1.000	0.800	0.800	0.000								
CSR90 IR-2	1.000	1.000	1.000	1.000	0.600	0.500	0.800	1.000	1.000	0.833	0.833	0.800	1.000	0.800	1.000	0.833	0.833	0.200	0.000							
FL-378	1.000	1.000	1.000	1.000	0.500	1.000	0.750	0.750	0.666	0.800	0.800	1.000	0.800	0.500	0.800	0.400	1.000	1.000	1.000	0.000						
FL-478	0.800	1.000	0.600	1.000	0.750	0.800	1.000	1.000	0.750	1.000	1.000	1.000	1.000	1.000	1.000	0.800	0.800	1.000	1.000	0.750	0.000					
IE71829	0.833	0.800	1.000	0.600	0.600	0.833	0.800	0.800	1.000	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	1.000	0.833	1.000	1.000	0.000				
IR 73571	1.000	1.000	1.000	1.000	1.000	0.800	0.750	1.000	1.000	0.800	0.800	0.800	1.000	1.000	1.000	1.000	0.800	0.750	0.800	1.000	1.000	1.000	0.000			
IR74095	0.833	1.000	0.800	1.000	0.600	0.833	0.600	1.000	0.750	0.833	1.000	1.000	1.000	1.000	0.833	1.000	0.833	1.000	0.833	0.800	0.800	0.500	1.000	0.000		
PBRC-30	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	1.000	0.833	0.666	0.800	0.833	0.800	0.666	1.000	0.666	0.800	0.666	0.800	1.000	1.000	0.600	1.000	0.000	
PBRC-37	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000	0.666	1.000	0.833	0.800	0.833	1.000	1.000	0.833	1.000	0.833	0.666	0.000

Genetic similarity analysis using UPGMA (Unweighted Pair Group Method of Arithmetic Means).

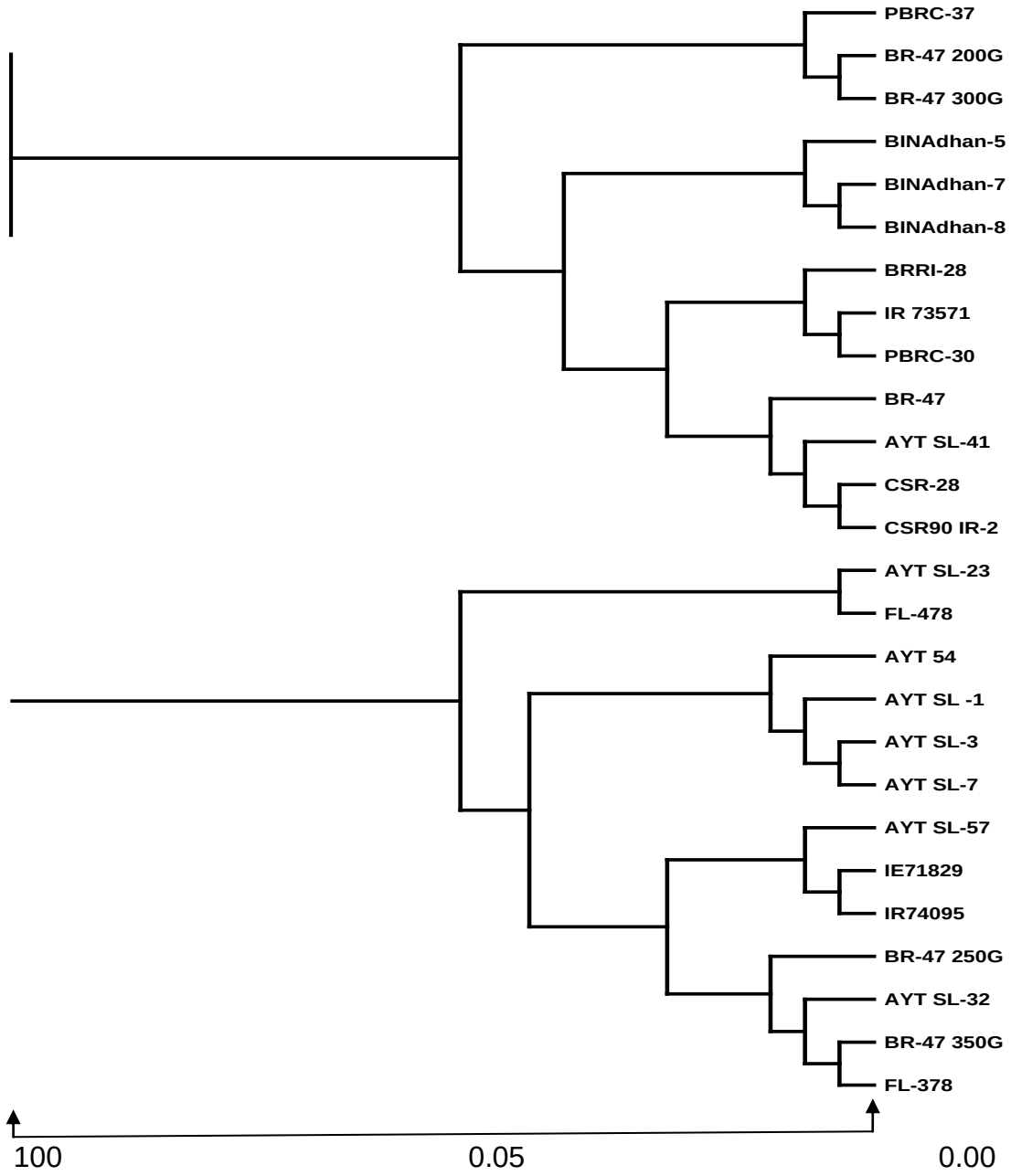

Fig 4. Dendrogram for 26 rice germplasms derived from a UPGMA cluster analysis.

Gene diversity

According to(Nei's, 1972) the highest level of gene diversity value (0.8994) was observed in loci RM10701 and the lowest level of gene diversity value (0.7743) was observed in loci RM152 with a mean diversity of 0.8544 (Table 6). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those detected higher number of alleles which revealed higher gene diversity.

Allele size range

The size variation between the smallest and the largest allele at a given SSR locus was correlated with the number of alleles per locus. Thus, RM152 presented the smallest allele size range (48 bp) and had eight alleles per locus, while RM11757 had the largest allele size range (176 bp) and a total of 9 alleles (Table 6).

PIC values

As a measure of the in formativeness of microsatellites, the PIC values ranged from a low of 0.7459 (RM152) to a high of 0.8908 (RM10701) and averaged 0.857 (Table 6). PIC values also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study.

Major allele

Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The frequency of the most common allele at each locus ranged from 15.38% (RM10701) to 37.51% (RM152) with a mean frequency of 24.15 (Table 7).

Genetic distance-based analysis

The values of pair-wise comparisons of (Nei's, 1972) genetic distance (D) between varieties were computed from combined data for the 6 primers, ranged from 0.200 to 1.000 (Table 8). Comparatively higher genetic distance (1.000) was observed between a number of accession or variety pair. Among them BINAdhan-7 vs. BR-47 250G; AYT-54 vs.PBRC-30, BINAdhan-8 vs. IR71829, CSR-28 vs. FL-378, and IR73571 vs. PBRC-37 was important.

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