Journal of Agricultural Science; Vol. 9, No. 6; 2017
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Assessment of Genetic Diversity in Wild Rice of Eastern India Using SSR Markers

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Received: November 8, 2016 Accepted: January 17, 2017 Online Published: May 15, 2017

doi:10.5539/jas.v9n6p239 URL: https://doi.org/10.5539/jas.v9n6p239

Abstract

Wild rice is an important reservoir of valuable and useful genes. *O. rufipogon* and *O. nivara* contain AA genome andare the progenitor of cultivated rice which makes them compatible with the cultivated rice for cross breeding to incorporate the genes for stress tolerance. SSR markers were used to assess the extent of diversity of 26 accessions of *O. rufipogon* and *O. nivara* collected from different districts of Orissa, West Bengal and Tripura. The Principal Co-ordinate Analysis (PCA) clearly indicates the clustering pattern and inter-relationships among different accessions. Mantel Z-testexhibitedacorrelation between cophenetic matrix and Jaccards' similarity coefficient in 26 accessions and 4 CRRI released varieties using 54 STMS (SSR) markers which showed significant correlation (r = 0.8249) between them. *O. nivara* accessions and *O. rufipogon* accessions were grouped different clusters. *O. nivara* collected from Midnapore is placed in different cluster. It is concluded that the SSR markers used were found to be equally informative for the genetic diversity study between and among the accessions of two wild species such as *O. rufipogon* and *O. nivara* collected from different locations of Orissa, West Bengal & Tripura. Highlysignificant morphological variations were also observed among *O. nivara* and *O. rufipogon* accessions.

Keywords: diversity, wildrice, morphology, SSR marker, Eastern India

1. Introduction

Rice is the world's most important cereal crop and is a primary source of food for more than half of the world's population (Khush, 1977). More than 90% of the world's rice is grown and consumed in Asia. *Oryza sativa* L. is a diploid species having 24 (2n = 24) chromosomes. It belongs to genus *Oryza* of family Poaceae. The genus *Oryza* includes 24 species of different genome (AA, BB, CC, DD, EE, FF, GG, HH) of which 22 are wild species (2n = 24, 48) and two species namely *Oryza sativa* and *Oryza glaberrima* are cultivated in Asia and Africa respectively. The Asian cultivated rice (*Oryza sativa*) originates in South and South-East Asia (Chang, 1985) and is grown world wide. Where as the African cultivated rice, *Oryza glaberrima*, is domesticated in West Africa. All the wild species played a significant role in rice breeding by contributing genes valuable to resistance for diseases, insect and pests and tolerant to abiotic stresses (Chang et al., 1975; Stich et al., 1989; Khush, 1977). A number of useful traits such as Cytoplasmic male sterility, resistant to Grassy stunt virus, Bacterial blight, Blast and Brown Plant blast hopper, have been introgressed from wild species into cultivated rice (Brar & Khush, 1997; Yuan, 1993). The wild species exhibit tremendous diversity in morphological traits. Besides these, it constitutes an exceptionally valuable genepool for rice improvement (Lu, 1996; Bellan et al., 1988; Zhong et al., 2000). Recently many diseases and pests resistant genes, high yielding genes and abiotic tolerant genes have been found in wild *Oryza* species (Khush et al., 1990; Jena & Khush, 1990; Brar et al., 1996).

All the AA genome species are the most accessible genetic resources because transfer the alien gene to *O. sativa* can be achieved through sexual hybridization (Jena & Khush, 1990). The fuller exploitation of the wild rice species gene poolessentially relies on the better understanding of the genetic diversity and relationship of the rice production (Sharma, 1983) through assessment of genetic diversity and relationship of rice and its wild relatives. *O. rufipogon* and *O. nivara* are the closely related species of Asian cultivated rice and considered as the progenitor of cultivated rice (Okha, 1988; Khush, 1997). *O. rufipogon* is perennial, photoperiod sensitive, largely

cross fertilized and distributed in South and South East Asian countries. It grows in swamps, and lakes year round. In contrast, *O. nivara* is an annual photoperiod insensitive, which is believed to be evolved from *O. rufipogon* because of habitat shift. It is distributed in Southeast Asia adapted to dry habitat (Vahugan & Morishima, 2003; Sang & Ge, 2007). The two species are cross compatible and exhibit narrow genetic differentiation (Oka, 1988; Zhu et al., 2007). *O. rufipogon* and *O. nivara* are treated as two ecotypes (Oka, 1988). Morphological variations are very high among the population and between the populations of *O. nivara* in Eastern India (Subudhi et al., 2004).

There is a remarkably rich diversity in cultivated rice and wild rice of Eastern India (Subudhi et al., 2002). However, a series of biotic and abiotic stresses continue to limit its productivity. Thus there is an urgent need to identify diverse sources of genes for tolerance to various stresses and broaden the rice gene pool. Wild rice with AA genome has been used as genetic resources to develop modern cultivars.

Measurement of morphological and biochemical characteristics has been commonly used method to arrive at an estimate of genetic diversity in parental stock material (Second, 1982). However, these characters can be influenced by environmental factors. Molecular markers may avoid many of these complications by looking directly at the genetic material itself. So in recent times Molecular markers represent a powerful and potentially rapid method for characterizing diversity.

2. Materials and Methods

Seeds from 12 accessions of *Oryza rufipogon*, 14 accessions of *Oryza nivara* and 4 CRRI released varieties were collected from NRRI Rice Gene Bank. The seeds were dried and germinated in petriplates by keeping seeds in a moist germination paper. Then the petriplates were kept in dark. Four days later the germinated seeds were transferred to individual pots. After three weeks, 3 grams of young leaves were collected from each accessions for genomic DNA isolation. The plants were maintained in pots. Some morphological observations were also recorded. The detailed passport information was given in Table 1. morphological data like Plant height, EBT/mt2, Leaf length, Leafwidth, Paniclelength, Panicleweight, Grain weight, Days to fifty percent flowering etc were recorded in selected five plants.

Young leaves were used for genomic DNA isolation. Total genomic DNA was extracted from young leaves by modified CTAB method (J. J. Doyle & J. L. Doyle, 1987). The quality of total DNA isolated was checked by adding 2 µl of 6X orange loading dye (Fermentas, USA) to 4 µl of isolated DNA. Six micro liter of this was loaded in a well of 0.8% (w/v) agarose gel (SRL) containing 0. 05 µg/ml of ethidium bromide PCR reaction, the optimum concentration (25 ng/µL) of the genomic DNA was used for the SSR markers taken for study. To bring the concentration of the sample genomic DNA into 25 ng/µl the sample DNA were diluted accordingly by adding TE buffer.

Microsatellite analysis was carried out using 60 (5 of each chromosome) mapped SSR markers distributed on all the 12 chromosomes (Temnykh et al., 2000) to assess the genetic relationship/variability of rice varieties (Table 3). The PCR reaction mixture contained 25 ng template DNA, 10 pmole of each of the primers, 0. 25 mM dNTPs (Fermentas), 1X PCR buffer (Biotool B&M Labs, Spain) (75 mM TrisHCl (pH 9.0), 50 mMKCl, 20 mM $(NH4)_2SO_4$ and 2 mM $MgCl_2$) and 1 unit of *Taq* DNA polymerase (Biotool B&M Labs, Spain) in a volume of 10 µl. The reaction mixture was initially denatured for 5 min at 94 ^oC, and, then, subjected to 35 cycles of 30 sec denaturation at 94 ^oC, 30 sec annealing at 55 ^oC and 1 min. extension at 72 ^oC; and a final extension for 10 min at 72 ^oC. Two microlitres of 6X loading dye was added to ten microliters of amplified products of each sample and was loaded on an ethidium bromide stained 2.5% agarose gel in 1X TBE buffer to separate the amplified fragments. The electrophoresis was done for about 3 hours at 100 volts.

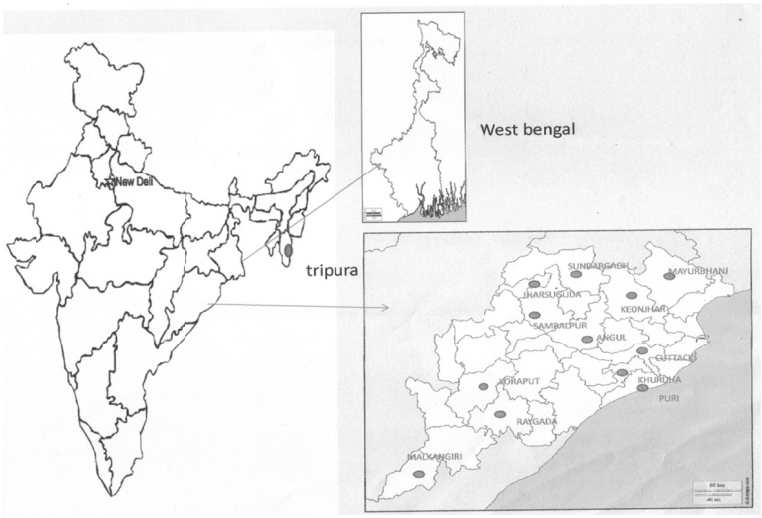


Figure 1. Collection sites of germplasm accessions

Table 1. List of *O. nivara* and *O. rufipogon* accessions and elite rice varieties used

SL. No.	Accession No.	species/varieties	District	State
1		*O. sativa* cv. Reeta.	CRRI	Orissa
2		*O. sativa* cv. Chandan.	CRRI	Orissa
3	100170	*O. nivara*	Puri	Orissa
4	100228	*O. nivara*	Ganjam	Orissa
5	100276	*O. nivara*	Sambalpur	Orissa
6	100288	*O. nivara*	Sundargarh	Orissa
7	100341	*O. nivara*	Sundargarh	Orissa
8	100316	*O. nivara*	Midnapur	West Bengol
9	100113	*O. nivara*	Malkangiri	Orissa
10	100134	*O. nivara*	Koraput	Orissa
11	100331	*O. nivara*	Sundargarh	Orissa
12	100329	*O. nivara*	Keonjhar	Orissa
13	100327	O. nivara	Keonjhar	Orissa
14	100578	*O. nivara*	Puri	Orissa
15	100522	*O. nivara*	Khurdha	Orissa
16	100293	*O. nivara*	Puri	Orissa
17	100150	*O. rufipogon*	Koraput	Orissa
18	100273	*O. rufipogon*	Anugul	Orissa
19	100342	*O. rufipogon*	Jharsuguda	Orissa
20	100398	*O. rufipogon*	Mayurbhanj	Orissa
21	100034	*O. rufipogon*	Rayagada	Orissa
22	100165	*O. rufipogon*	24-Pragana	West Bengal
23	100343	*O. rufipogon*	Jharsugada	Orissa
24	100334	*O. rufipogon*	Sundargarh	Orissa
25	100530	*O. rufipogon*	Dugli	Tripura
26	100528	*O. rufipogon*	Dulki	Tripura
27	100531	*O. rufipogon*	Matarbari	Tripura
28	100346	*O. rufipogon*	Puri	Orissa
29		*O. sativa* cv. Geetangali.	CRRI	Orissa
30		*O. sativa* cv. Vandana.	CRRI	Orissa

3. Result and Discussion

3.1 Morphological Study

The data on eight morphological characters like plant height, ear bearing tillers, leaf length, leaf width, panicle length, panicle weight, 100 grain weight and days to fifty percent flowering etc. were recorded in 5 well grown plants for each genotype to assess the phenotypic variation of 26 wild rice accessions. The analysis of variance was given in Table 2. The plant height varied from 54.3 cm. (ac100228) to 177 cm (ac100165). Similarly, Ear bearing tillers ranged from 5.0 (ac100331) to 13.5 (ac100343). Leaf length ranged from 19.6 cm (ac100331) to 63.7 cm (ac100334). Panicle length is highest in ac100398 (31.6 cm) and lowest in ac100329 (14. 4 cm). Panicle weight varied from 0.75 g (ac100334) to 2.35 g (ac100331). Days to fifty percent flowering varied from 48.5 days (ac100341) to 144 days (ac100342). CV(%) is highest in ear bearing tiller (16.04) and lowest in DFF (2.46). Yibo et al. (2010) and Subudhi and Nayak (2002) also observed large variation in morphological traits among *Oryza rufipogon* population.

Table 2. Source of variation of 8 morphological traits in 26 wild rice accessions

Source of variation	DF	PH (cm)	Mean sum of squares					GW (g)	DFF (days)
			EBT/Pl.	LL (cm)	LW (cm)	PL (cm)	PW (g)		
Replication	1	73.92	7.69	2.32	.0008	2.04	.000	.443	50.01
Treatment	25	3952.17	13.71	405.24	.064	68.16	.254	.062	2721.9
Error	25	6.44	2.01	3.56	.009	.769	.032	.031	5.61
Min		54.3	5.0	19.6	0.45	14.4	0.75	0.85	48.5
Max		177.0	13.5	63.7	1.05	31.6	2.35	1.45	144
Average		111.9	8.82	39.89	0.71	23.07	1.36	1.16	96.45
CV(%)		2.27	16.04	4.7	13.2	3.8	13.22	14.98	2.46
SE		.704	.393	.524	.027	.243	.050	.049	.657
P value		.0023	.061	.427	.766	.115	1.00	.0009	.0063

Note. PH = plant height; EBT/PL = ear bearing tiller; LL = leaf length; LW = leaf width; PL = panicle length; PW = panicle weight; GW = grain weight; DFF = days to fifty percent flowering.

3.2 Statistical Analysis

The data were scored as 1 for the presence and 0 for the absence of the band for each primer-variety combination for SSR analysis. Resolving power of the primer/primer combination was calculated as per Prevost and Wilkinson (1999) is:

$$Rp = \Sigma IB \text{ [IB (band informativeness)} = 1 - [2 \times (0.5 - P)] \quad (1)$$

Where, P is the proportion of the 30 varieties containing the band.

The Primer Index was calculated from the polymorphic Index. The Polymorphic Index (PIC) was calculated as $PIC = \Sigma P_i^2$, where, P_i is the band frequency of the ith allele. Here, the PIC was considered to be $1 - p^2 - q^2$, where, p is the band frequency and q is no band frequency (Ghislain et al., 1999). PIC value was then used to calculate the SSR Primer Index (PI). PI is the sum of the PIC of all the markers amplified by the same marker. The term polymorphism information content (PIC) refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency. In the present study, PIC value of a marker was calculated according to a simplified version of Anderson et al. (1993):

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2 \quad (1)$$

Where, P_{ij} is the frequency of the jth allele for the ith marker, and summed over n alleles.

Jaccard's coefficient of similarity (Jaccard, 1908) was measured and a dendrogram based on similarity coefficients generated by unweighted pair group method using arithmeticaverages (UPGMA) (Sneath & Sokal, 1973), and the SHAN (Sequential AgglometricHierarchial and nested) clustering was obtained. The entire analysis was performed using the statistical package NTSYS-pc 2.02e (Rohlf, 2000).

3.3 Molecular Study

Table 3. Sequence and Chromosome position of SSR (STMS) markers screened

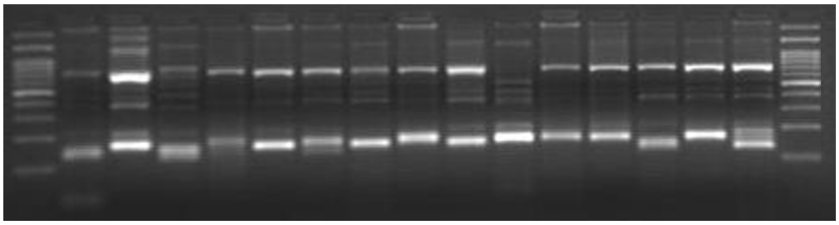
S. No.	Marker	Forward Sequence	Reverse Sequence	Chr #	Annea-ling temp (^{o}C)
1	RM5443	CTCATTGGCACATCTACATACAGG	TCCAACTAAGCAGAAGAACTAGGG	1	55
2	RM11278	ACTTCTTGTAGCACTGCACCTTCG	CCTCGGCAACTGCTTCAAGG	1	55
3	RM11229	TGACAGAAACAAAGCGGAAGG	TCCAAACCGCTATTCTTGTAGC	1	55
4	RM11111	CTGTTGTCGGTTACACCTTGTGC	CCACTCCTCTATAGCGTCATTCACC	1	55
5	RM11258	GCTCCACCATTCATCCATACAGC	ATTCGATTGGTTCCTTGGCTTCC	1	54
6	RM12349	CCGATTAGCGATTGATATGGAGTAGG	AGTGCACAGCCATGGAATTATGC	2	57
7	RM13097	GGGCTTAAGGACTTCTGCGAACC	AGCGATCCACATCATCAAATCG	2	57
8	RM13260	AGTCCAAGAAAGGCACGAGAGG	CTGCATCGAAGAAGAAGAAGAAGC	2	54
9	RM341	GGCTCGGCTAGGCTTGATCC	GGGAGGTCGAGATGGGAACC	2	54
10	RM13357	CATAAAGGGACCCACTTGTCAGC	TGATGTCTGGTCCACAGTCTGC	2	57
11	RM14320	CACCTGTAAATTAGGACACTGG	CAGTGTACTTTGAACTGCCTAGC	3	51
12	RM14860	GGAAGGTGATTTCATCCGGTAGC	TGGCATGTTTAATGCTGGTTCG	3	54
13	RM14981	GGCGAGCAGAAGTATAATCCAGAAGG	CGCTTGTGGCTTACTGGCTTGG	3	55
14	RM15981	GGTTAAAGGGAGGACACCACTCG	TCTAGCCAGGCATGACAAGAACC	3	55
15	RM15809	AAAGCTGCGACGAACACGAACG	CGCCGCAGCAGAGAAGAGAAGG	3	55
16	RM16770	AACCGGCCATGCCAGAGAGG	CCAAATCCTATCCGCCACACACC	4	51
17	RM16291	GTACACACCCACATCGAGAAGC	TCCATGGATATACGAGGAGATGC	4	51
18	RM17201	GATCGTTGCTGCTTTCAATGAGG	AGTGTTTCACCTTGGACCCATGC	4	54
19	RM17008	TTACCTTCGATTAGCTGCTGTTGC	ATTCCTTGCATTACAGACGGTAGC	4	54
20	RM17480	GAGTTCGTCCCTGACAAACAGAAACG	GTGAGCGAGCGAGTGAGTGAGC	4	54
21	RM18065	CGATGGTGAGTGGTGATTCATGC	ATCATCCGCGCATTAGCATTTCC	5	54
22	RM18691	GCAGTTCGTTGTGGAGGAACACC	ATCGGCCACCCAAATCTTAATGG	5	54
23	RM19029	AAATATCTATCGGCCTCTCCAAGC	GGAGGAATCGAACCAGAGAAGC	5	54
24	RM17702	TAAACTGCTCTTGCCTCTTCTCC	TCATTCCAATCCAGTCATCTCC	5	55
25	RM18360	TCGAGACTGATCGGAGTTTAGGC	CGCTCCTCCCTAACACCTCTACG	5	55
26	RM19255	TTAAGCTAGGGAATCAGCGGTTAGC	GGAGTTGCAGTGTGGTGTGTGG	6	54
27	RM19844	CGTTCGAGCAGAACCATCTACC	CCTCTTCCGCTCCACTCTCC	6	54
28	RM20615	TTACACCAGGCTACCCAAACTCG	TTGCTGAGTTCCCTCGTCTATCC	6	54
29	RM20368	CAAGAATCAAGAGCCGAGAGTCC	TCTTCTGTACGGTTTCTTGGTTGC	6	54
30	RM21024	ATTAAGCTACTGTCTGCCTCCTTCG	GCTTCGTTTCAGGTGGTCAGG	7	53
31	RM21258	TATCATTCCGGTCCAAAGTGTCG	TCCGGTCCAAAGTCTCATTTGC	7	53
32	RM21559	GTATAGCCAACCAAGCAAGATAGC	GATGCCTAGACACACATGTAAGC	7	53
33	RM21776	TCGGGTATAATTATCGCAGCACACG	ATGGATGGTACGAGGACGAGAGC	7	54
34	RM22252	AAGGAGAAGTTCTTCGCCCAGTGC	GCCCATTAGTGACTGCTCCTAGTCG	8	55
35	RM22571	TCCTCCTCCACCTCAATCACACC	CCGAGCTCGCCATTAGCTTGC	8	55
36	RM22914	GGAGCAGCTAAGGCAGATAAGAGG	GCCTTCATGCTTCAGAAGACAGC	8	53
37	RM22825	AGCACATCACAAACCTACCCTACC	CCTAATTAATCCCGCGGAACC	8	53
38	RM23578	AGCGATTCAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC	8	54
39	RM24683	CAGTGGCGTGGAGAGAAATTTGG	CTCACCTGCGACAGCAAGATCG	9	54
40	RM24260	GATCTCTCACCCACATGCCTAGC	TCCCATCTTGATCGATCTCTTCG	9	54
41	RM24559	AGTTGAGTGGCAAACCACAGAGC	CTTCACTTGGGTTTGGGTGATGG	9	54
42	RM24717	CCTCACTCCCGTACAGTTGAACC	TAAGGCCATTCCGTTGATGTGG	9	55
43	RM25368	TATAGTTAAGGGAGCCACGCAAGC	CCACCTCGTAAGAACATGGAGAACC	10	55
44	RM25712	TCTCCCTATTCCCGTGTAAATCG	CCCGATGATCGATTGTACCTAGC	10	55
45	RM25544	TCAGTTCGGGAAGTCAGATTCCTACC	CCAGTCTTACGGAGGTGCTCATAGG	10	55
46	RM25817	GCCTCGAATCAACCAAATAGTGG	GGGCTGAGACCTTGTGGTATGG	10	54

47	RM26033	AAGAGGACCTCGAGGATGTACCG	CGTCAGCCTGTCTTGTTGTACTCG	11	55
48	RM26269	GGAGGTAGGGAAATCAGGTGAGG	GTGCACGTGACCATAAACACTCC	11	55
49	RM26579	GCGGACACACCAGAGAATAAGC	GTGCTGTCCTGTCCTTGAATCC	11	55
50	RM27201	GCCTACTGGTCCAAGGTGAAGC	CAAGTAGACGTCGTGGAACATGG	11	55
51	RM27507	TGTATAGCCCGGAAGTATGATCC	TCTGGTCTCGTCTCTCATGTGC	12	54
52	RM27635	CCAAACTATCAGCGACATGAACG	CTCTGTTGTTTGGGAGACTGTGC	12	54
53	RM27955	AATGGTGCTTACTCACAGCAATGG	TTCATGAGCTGTGCCTGTTGG	12	54
54	RM28464	CTATATCCGCACCAATGGAAACC	TAGGAATTGCGACGAGTTTGTGG	12	54
55	RM27487	CCAAGCACCACATTTGGTTTCC	AACCTTGCTCAGCAGGACAGC	12	55

To visualize the genetic diversity among 26 accessions and four CRRI released varieties in detail, PCA was performed with 55 SSR markers. The description of the data was done using three dimensions. This technique helps in converting a set of variables into a few dimensions using which the genotypes under study can be depicted in a two or three dimensional space (Ludwig & Reynolds, 1988) so that the variations of several individuals will be condensed into a set of limited axes. Such a graphical analysis helps in identifying the individuals that tend to cluster together. Principal Co-ordinate Analysis was also performed using the NTSYS-pc 2.02C Software.

The Mantel 'Z' test was performed to study the efficiency of marker technique (Mantel, 1967).

RM-314



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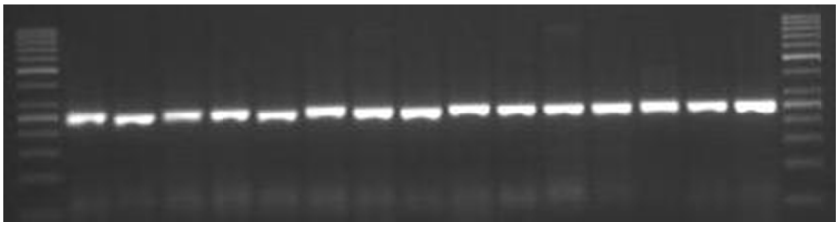

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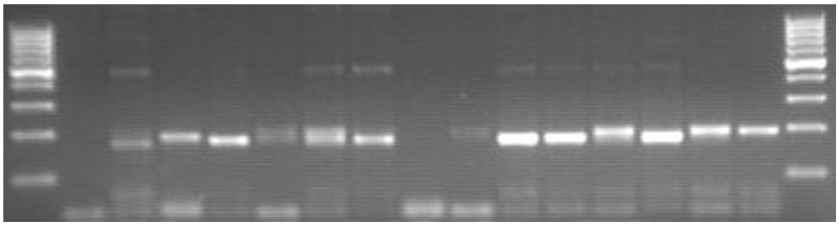

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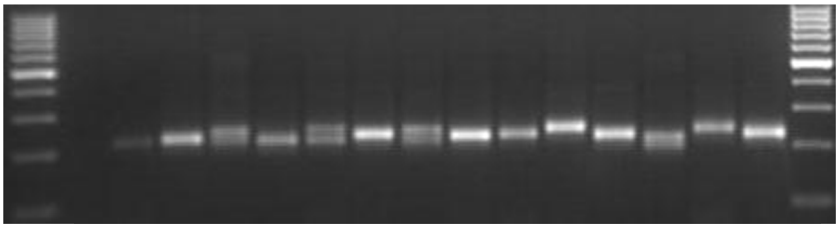

Figure 2. Gel profile showing the amplification of SSR marker in different primers

Analysis of variance revealed significant differences among the genotypes for all traits, indicating presence of high variability. A total of 55 STMS markers were used to analyse the DNA samples of 26 accessions in wild rice along with 4 released elite rice varieties out of which only 54 STMS (SSR) markers showed best amplification and reproducibility; from which total one hundred sixty five bands were amplified (Table 4). The DNA profiles obtained by STMS markers are represented (Figure 2). The marker wise results for total bands, polymorphic bands, monomorphic bands, unique bands, resolving power, marker index and PIC are presented in Table 4.

Table 4. Details of the SSR markers used for the study

S. No.	Primer	Approx. Frag. size	TB	PB	MB	UB	RP	MI	PIC
1	RM11219	352-395	2	2	0	0	2.067	0.499	0.711
2	RM5443	120-140	2	2	0	0	2.000	0.500	0.660
3	RM 22914	150-180	3	3	0	0	2.267	0.470	0.731
4	RM11278	250-280	4	3	0	1	2.333	0.413	0.994
5	RM11111	460-500	2	2	0	0	2.000	0.500	0.749
6	RM24260	220-260	3	3	0	0	1.667	0.401	0.931
7	RM 341	130-170	6	5	0	1	3.933	0.441	0.769
8	RM 11258	200-300	4	4	0	0	3.067	0.473	0.593
9	RM 12349	280-295	5	4	0	1	2.067	0.328	0.815
10	RM 13097	120-170	3	1	1	1	4.000	0.444	0.532
11	RM 13260	120-190	4	4	0	0	3.133	0.477	0.524
12	RM 24683	140-198	3	3	0	0	2.133	0.458	0.793
13	RM 22571	90-140	3	3	0	0	2.867	0.499	0.639
14	RM 13357	320	1	0	1	0	2.000	0.000	0.000
15	RM 14981	500	1	0	1	0	2.000	0.000	0.000
16	RM 15809	200-220	3	3	0	0	2.000	0.444	0.853
17	RM 25368	180-210	2	2	0	0	2.133	0.498	0.644
18	RM 25712	260-294	4	3	0	1	2.067	0.383	0.892
19	RM 25817	200-290	2	1	0	1	2.000	0.500	0.532
20	RM 26033	300-350	3	3	0	0	2.467	0.484	0.719
21	RM 26269	90-200	3	3	0	0	2.400	0.480	0.851
22	RM17702	160-190	3	3	0	0	2.400	0.480	0.831
23	RM 19255	120-140	4	4	0	0	2.667	0.444	0.861
24	RM19844	175-400	2	2	0	0	3.667	0.153	0.159
25	RM20615	280-300	3	2	0	1	1.333	0.346	0.986
26	RM21024	290	3	2	0	1	1.333	0.346	0.877
27	RM21258	300	4	4	0	0	4.800	0.480	0.473
28	RM21559	120-200	3	3	0	0	3.133	0.499	0.671
29	RM18360	290-300	2	1	0	1	2.000	0.500	0.532
30	RM27487	160-180	3	2	1	0	4.133	0.429	0.480
31	RM27507	130-180	4	3	0	1	1.867	0.358	0.964
32	RM27635	700-800	5	3	0	2	2.867	0.409	0.819

33	RM27955	200-240	2	2	0	0	1.933	0.499	0.704
34	RM22252	180-195	2	2	0	0	1.733	0.491	0.802
35	RM16770	95-100	3	3	0	0	3.333	0.494	0.633
36	RM16291	400	2	1	1	0	2.133	0.498	0.498
37	RM14860	195	1	1	0	0	0.733	0.464	0.866
38	RM28464	320-500	3	1	0	2	0.667	0.198	0.999
39	RM14320	380-450	2	2	0	0	0.333	0.153	0.993
40	RM17008	160-180	3	2	0	1	1.200	0.320	0.906
41	RM17201	150-200	6	4	0	2	4.133	0.452	0.997
42	RM18065	520	4	3	0	1	5.400	0.439	0.219
43	RM20368	330-350	4	4	0	0	1.333	0.278	0.977
44	RM27201	190	6	4	0	2	3.267	0.396	0.731
45	RM21776	140-180	3	1	0	2	2.000	0.444	0.564
46	RM25544	100-195	3	3	0	0	2.000	0.444	0.877
47	RM24717	150-220	6	5	0	1	2.133	0.292	0.997
48	RM26579	80-90	2	2	0	0	2.000	0.500	0.616
49	RM22825	90-100	2	2	0	0	2.000	0.500	0.740
50	RM23578	100-120	4	4	0	0	3.133	0.477	0.719
51	RM15981	200-210	3	3	0	0	2.133	0.458	0.900
52	RM17480	190-200	2	1	0	1	1.267	0.433	0.819
53	RM18691	480-490	1	0	1	0	2.000	0.000	0.000
54	RM24559	200	2	2	0	0	0.667	0.278	0.962
Total		**165**	**135**	**6**	**24**	***2.340**	***0.401**	***0.706**	

Fifty four STMS markers produced a total of 165 bands, out of which 135 bands (81. 81%) were polymorphic in nature. The maximum number of total bands (6) was amplified with RM341, RM17201, RM27201 and RM24717 while RM13357, RM14981, RM14860 and RM18691 produced the lowest number (1) of total bands. The amplicons were observed in the range of 80 to 800 bp. The highest and lowest amplicon size was found in RM27635 and RM26579 respectively. The highest number of polymorphic bands (5) was produced with RM 341 and RM24717 markers, whereas the lowest number (0) was amplified in RM13357, RM14981 and RM18691 markers. The Resolving Power (RP) of the markers varied from 0.333 to 5.400, while the marker index varied from 0.153 to 0.500. Best Resolving Power was observed in the marker RM18065. The maximum Marker Index (MI) 0.500 was observed with the markers RM5443, RM11111, RM25817, RM1127, RM18360, RM26579and RM22824 and the minimum (0.153) was in RM19844 and RM14320. The maximum PIC value (0.999) was observed with the marker RM28464 followed by 0.997-900 in RM17201, RM 20368, RM24717, RM20615, RM27507 and RM 15981 while RM19844 showed the minimum value (0.159)

Jaccard's similarity coefficient showed that accession no. 100578 (sl. no. 14) (*O. nivara*; collected from Puri) and accession no. 100522 (sl. no. 15) (*O. nivara*; collected from Khurdha) were most closely related with a similarity value of 0.775 followed by accession no. 100170 (sl. no. 3) (*O. nivara*; collected from Puri) and accession no. 100316 (sl. no. 8) (*O. nivara*; collected from Midnapur) with similarity value of 0.186. The matrix value ranged from 0.186 to 0.775.

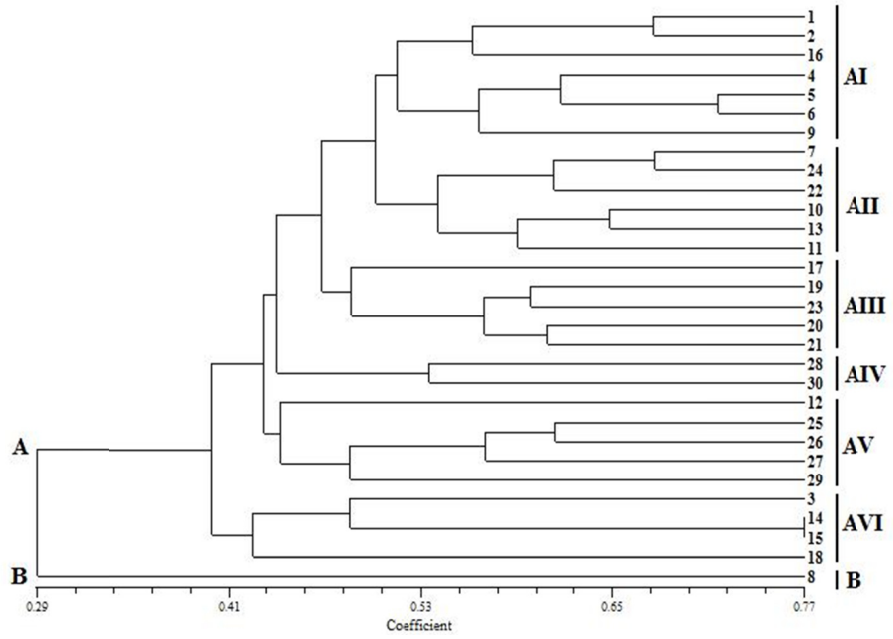

Figure 3. Dendogram depicting genetic relationship within and among 26 accessions of two wildrice species & CRRI released varieties based on SSR markers

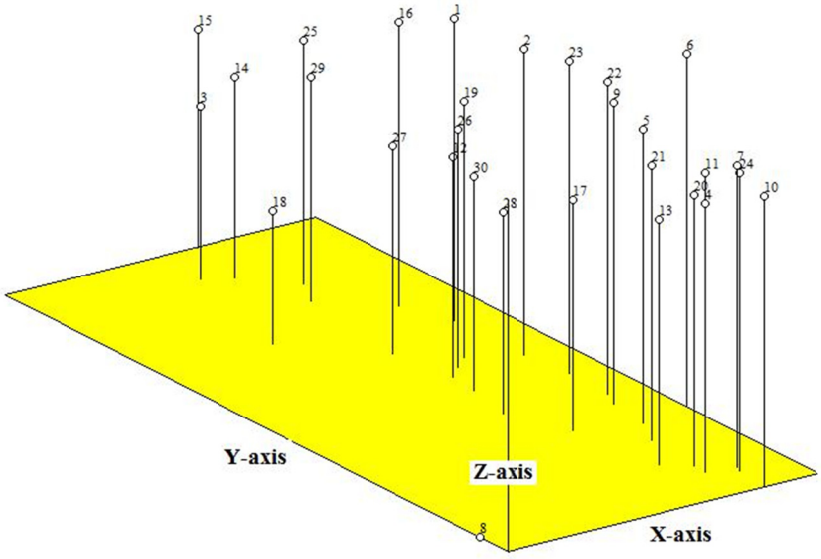

Figure 4. Principal coordinate analysis of 26 accessions and 4 CRRI released varieties based on SSR mark

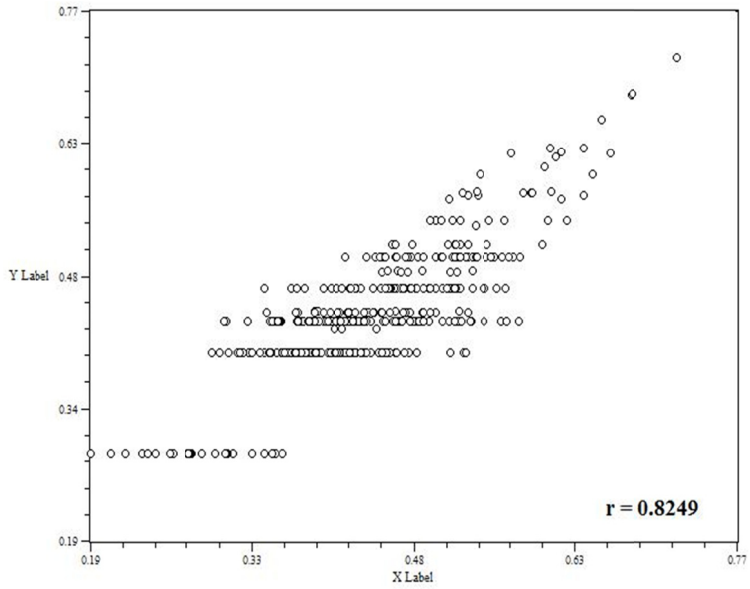

Figure 5. Mantel Z correlation in 26 accessions and 4 CRRI released varieties based on 54 SSR markers

Dendrogram constructed indicates two broad clusters. *O. nivara* collected from Midnapore is in one cluster indicating completely different genes. Other group is divided in six sub groups. The groups 1 and 2 includenine *O. nivara* accessions with two accessions of *O. rufipogon* and two cultivated rice. The sub group 3, 4, 5 and 6 includes *O. rufipogon* accessions with four *O. nivara* accessions and two cultivated rice. Cultivated rice are grouped differently due to different parents used.

The reason behind cross grouping may be that *O. nivara* and *O. rufipogon* are known to out cross to a varying degree (5-25%) unlike the cultivated species, which are predominantly self-pollinated. Inter specific hybridization between cultivated rice and *O. nivara* or *O. rufipogon* in natural habitat have been reported (Oka & Chang, 1989). Sympatric occurrence of *O. nivara* and *O. rufipogon* and Wild rice habitats near rice fields facilitate the exchange of genes through natural out crossing. But SSR markers were successfully employed for genetic diversity analysis in *O. nivara* and *O. rufipogon* accessions (Sarala et al., 2003; Yibo et al., 2010).

4. Conclusion

It can be concluded that SSR marker has the discriminatory power to reflect genetic diversity between and among the 26 accessions of two wild rice species such as *O. rufipogon* and *O. nivara* collected from different places of Orissa, West Bengal and Tripura including 4 released elite rice varieties.

Based on polymorphic features among the accessions of two wild rice species based on SSR study, it may be recommended that any future conservation plans for these species should be specifically designed to include representative accessions with the highest genetic variation for both *in situ* conservation and germplasm collection expeditions. The unique bands that could be identified are likely to provide tags for future genetic improvement as well as in authenticating the genotypes. Further investigation using more markers may be helpful for the accuracy and resolution of genetic diversity.

The level of genetic diversity detected in the present study could provide valuable inputs for trait mapping and marker assisted selection, thus paving the way for marker assisted improvement and related genome analysis of these two wild rice species. SSR markers along with morphological characters could now be used as coherent tools for the development of core collection of *two* wild rice species.

The SSR markers should complement one another during genetic identification, by coding different regions of *O. rufipogon* and *O. nivara* genome.

Need for Conservation: The diversity of *Oryza nivara* and *Oryza rufipogon* is very rich in Eastern India. These two species are having many valuable genes for abiotic stresses. These areas are also poorly explored and characterized. In recent times, due to habitat destruction, erosion of local population is very high and alarming.

So it is high time to conserve the local population of wild rice for future hybridization programme and gene transfer.

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