Plant Omics Journal POJ

POJ 6(2):121-127 (2013) ISSN:1836-3644

Wild rices of Eastern Indo-Gangetic plains of India constitute two sub-populations harbouring rich genetic diversity

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Abstract

Analysis of variability and genetic structure of wild rice populations is important for the management and conservation of the valuable genetic resources. To better understand the relationships among wild rice accessions, we analyzed a subset of germplasm collected from the eastern Indo-Gangetic plains of Uttar Pradesh and Bihar. Thirty-five wild rice accessions were characterized for fourteen morphological traits and genotyped using 25 genome wide SSR markers. The accessions showed significant phenotypic variation for all the traits analyzed. Analysis of SSR markers revealed average 2.4 alleles per locus with PIC values ranging from 0.51 to 0.90 with an average of 0.79. Cluster analysis and principal component analysis clearly demarcated the wild rice accessions into two main groups representing *Oryza rufipogon* and *Oryza nivara*. The high level of genetic diversity found in wild rices of this region suggests that it is a valuable resource that should be conserved for utilization in rice breeding programs.

Keywords: Wild rice, Genetic diversity, SSR markers, Morphological traits, Eastern Indo-Gangetic plains.
Abbreviations: IGP: Indo-Gangetic plains, PCA: Principal component analysis, PIC: Polymorphism information content.

Introduction

The genus *Oryza* consists of twenty two wild and two cultivated species (*O. sativa* and *O. glaberrima*). Asian cultivated rice *O. sativa* (2n = 24) is the world's most important food crop and is primary food source for more than one third of the world's population. Rice accounts for 35 to 60% of the calories consumed by 3 billion Asians (Khush, 2005). To meet the future demands of food for ever increasing population, there is an urgent need to enhance the productivity of this crop that seems to have reached a plateau (Khush, 2003) due to a narrow genetic base of the modern rice cultivars. It is essential to search for the new traits and genes in the wild rice germplasm and landraces of rice to combat this problem. Although there are many sources of natural variation, wild relatives of cultivated crop species are increasingly recognized as a valuable repository of useful allelic variation for crop improvement. Wild species of rice are reservoir of many useful genes but a vast majority of these genes remain untapped because it is often difficult to identify and transfer these genes into cultivated *indica* rice. Historically, wild rice species have provided many valuable traits such as disease and pest resistance and cytoplasmic male sterility (Brar and Khush, 1997). *O. rufipogon* Griff. and *O. nivara* Sharma *et* Shastry are the most closely related species to Asian cultivated rice (*O. sativa*) and are considered its progenitors (Oka, 1988; Khush, 1997). The geographic range of *O. sativa* and *O. rufipogon* overlap throughout Asia. *O. rufipogon* further evolved and formed a complex which includes *O. nivara* and *O. rufipogon*. *O. rufipogon* is perennial, photoperiod sensitive, largely cross-fertilized, and widely distributed in South and Southeast Asia, Southern China, Papua New Guinea and Northern Australia. It grows in areas with year round water, such as swamps and lakes. In contrast, *O. nivara* is an annual, photoperiod insensitive and predominantly self-fertilized species which is believed to have evolved from *O. rufipogon* because of habitat shift. This species is restricted to South and mainland Southeast Asia and adapted to seasonally dry habitat (Vaughan and Morishima, 2003; Sang and Ge, 2007). Because of their important role in providing beneficial genes for rice breeding *O. rufipogon* and *O. nivara* have long been the subject of extensive taxonomic, phylogenetic and population studies using a variety of approaches (Khush, 1997; Vaughan et al., 2003). The two species are cross compatible and exhibit narrow genetic differentiation (Oka, 1988; Lu et al., 2002; Zhu et al., 2007). Consequently, *O. rufipogon* and *O. nivara* have sometimes been treated as two different ecotypes of the same species (Oka, 1988; Cheng et al., 2003;Vaughan and Morishima, 2003).

Indian subcontinent can be divided broadly into three main geographical regions: the Himalayas in the far north, the Indo–Gangetic Plain in the north-central area and the southern Peninsula. The Indo-Gangetic plain (IGP) is one of the most intensively farmed zones of the world and is crucial for the food security (Thakur and Pandey, 2009). The eastern IGP is endowed with a great diversity of wild rice growing in its natural habitats. In recent years, a considerable amount of information about the genetic structure within and among natural populations of *O. rufipogon* and *O. nivara* has been obtained (Kuroda et al., 2007; Zhou et al., 2008), but *O. rufipogon* and *O. nivara* germplasm present in eastern IGP is not well characterized. Recently, a large number of wild rice germplasm were collected from the major rice growing areas

of eastern IGP of Uttar Pradesh and Buxar district of Bihar. The understanding of the extent and type of genetic variation of wild rice populations at the molecular level will help for the development of appropriate strategic collection, conservation and utilization of wild rice of this region. Molecular markers are powerful tools for studying genetic diversity and population differentiation of plant species (Parker et al., 1998). Microsatellite loci, also known as simple sequence repeat (SSR) is frequently used as multi allelic co-dominant markers in plants to study genetic diversity and evolutionary relationships. Past studies have demonstrated applicability of SSRs for determining both intra- and inter-population genetic structures (Kuroda et al., 2003, 2005). Therefore, the objective of the present study was to analyse the genetic diversity among *O. rufipogon* and *O. nivara* germplasm collected from different districts of eastern IGP using SSR markers.

Results and discussion

The development of high-yielding, disease and pest resistant rice cultivars will be aided greatly by effective and efficient utilization of variability present in the wild rice germplasm. The eastern IGP is endowed with a great diversity of wild rice. The knowledge of genetic diversity in the *O. rufipogon* and *O. nivara* populations of eastern IGP is fundamental to the strategic collection expeditions and conservation of germplasm.

SSR diversity and genetic relationship among wild rice accessions

Panicles of 35 different wild rice genotypes were collected from natural habitats of Eastern Uttar Pradesh and Buxar district of Bihar, India (Table 1; Fig.1) and grown in a growth chamber for DNA isolation. The 25 polymorphic SSR markers were applied to the 35 wild rice accessions which generated 60 different bands (Table 2). The number of alleles generated per marker locus varied from 2 to 3 with an average of 2.4 alleles per marker. The size of amplicons varied between 140 bp to 250 bp. Electrophoretic patterns of SSR markers RM-206, RM-152 and RM-264 are shown in Fig 2. The PIC values provide an estimate of the discriminating power of a marker. The PIC value for the 25 SSR markers ranged from 0.51 (RM-20) to 0.90 (RM-38) with an average of 0.79. The PIC values of the SSR markers was higher as compared to earlier study on genetic diversity in *O. nivara* germplasm by Juneja et al. (2006), which ranged from 0.41 to 0.80 with an average of 0.64. Jaccard's similarity matrix was used to generate a dendrogram to obtain the clustering of genotypes (Fig 3). The similarity coefficient ranged from 0.02 to 1.00. The highest similarity coefficient (1.0) was observed between accessions NKSWR-54 and NKSWR-55 collected from the same location from the Ballia district of Uttar Pradesh, while the lowest (0.02) was between NKSWR-37 and NKSWR-32 & NKSWR-34. The accessions NKSWR-54 and NKSWR-55 were indistinguishable from each other on the basis of SSR markers. Interestingly, these two accessions were morphologically distinct therefore could not be duplicates. Earlier, SSR markers were successfully employed for genetic diversity analysis in *O. nivara* and *O. rufipogon* accessions (Sarla et al., 2003, Yibo et al., 2010). The dendrogram constructed based on the similarity coefficients grouped the genotypes into two major clusters corresponding broadly to *O. rufipogon* and *O. nivara*. The larger cluster, cluster I consisted of 20 accessions out of which 15 accessions belonged to *O. rufipogon*. The cluster II consisted of 15 accessions including 13 accessions of *O. nivara*. Grouping of five *O. nivara* accessions in *O. rufipogon* cluster and two *O. rufipogon* accessions in *O. nivara* cluster was observed. The reason behind cross-grouping may be that *O. nivara* and *O. rufipogon* are known to outcross to a varying degree (5–25%), unlike cultivated rice genotypes which are predominantly self-pollinated. Interspecific hybridization between cultivated rice and *O. nivara* or *O. rufipogon* in natural habitats has been reported (Oka & Chang, 1959, 1961). Sympatric occurrence of *O. nivara* and *O. rufipogon* in nearby wild rice habitats near rice fields facilitates the exchange of genes through natural outcrossing. The PCA analysis carried out using SSR data is shown in Fig. 4. The eigen value obtained from the first three principal components cumulatively accounted for 62.72% of the total variation, in which 36.45% is accounted for by the component 1 and 21.76% by the component 2. The PCA analysis also resulted in separation of genotypes in two groups broadly belonging to *O. nivara* and *O rufipogon*. The PCA analysis supports the grouping as observed by UPGMA based dendrogram. Two accessions namely, NKSWR-48 and NKSWR-53, belonging to *O. rufipogon* and one accession namely, NKSWR-25, belonging to *O. nivara* were separated from the two groups. Since *O. rufipogon* and *O. nivara* were sympatric in the eastern IGP, therefore their outgrouping from their original population may be due to exchange of genes through natural outcrossing.

Morphological variations in wild rice population

Total fourteen morphological and yield related traits were recorded for assessment of phenotypic diversity among the 35 wild rice accessions. The germplasm used in the present study showed significant phenotypic variation for all the characters analyzed. The basic statistical analysis of the results with respect to yield and yield-associated traits are presented in Table 3. There was a wide variation in seeds and panicles color, shape and size (Fig. 5, Table 3). Maximum number of effective tillers per plant was recorded for NKSWR-4 (8.19) while minimum number of effective tillers per plant was for NKSWR-48 (2.54). Days to maturity was found maximum in NKSWR-99 (157 days), while minimum in NKSWR-57 (129 days). A large variation was observed in plant height which ranged from 128 cm (NKSWR-46) to 210 cm (NKSWR-53). Variation in panicle length was also very large ranging from 17.72 (NKSWR-4) to 28.50 (NKSWR-53). A large variation in filled grain per panicle and total grain per panicle was observed ranging from 19.10 (NKSWR-54) to 231.90 (NKSWR-48) and 54.61 (NKSWR-82) to 267.29 (NKSWR-48) respectively. Spikelet fertility was recorded maximum in NKSWR-48 (86.77) and minimum in NKSWR-54 (23.44). The grain yield per plant was found highest in NLSWR-48(36.99 g) followed by NKSWR-75 (35.36 g) and NKSWR-64 (35.26 g). Significant variation was also observed for days to 50% flowering, leaf length, leaf width, test weight, kernel length and kernel width. The CV values for all the quantitative traits were quite high, being >80% for the effective tillers per plant, panicle length, filled grain per panicles and total grain per panicle (Table 3). The CV values for rest of the quantitative traits also indicated a high level of variation (i.e.>25%) except kernel length (16.70). In a previous study Yibo et al. (2010) also observed large variation for 13 morphological traits among *O. rufipogon* populations. Thus, wild rice accessions display numerous distinctive morphological traits, which may prove useful in the selection of donor lines for introgression

Table 1. List of 35 wild rice accessions used in the present study, their collection sites and species level.

Serial no.	Accession no.	Collection site (village, block, district)	Species
1.	NKSWR-1	Bhainsa, Sewapuri,Varanasi	*O. nivara*
2.	NKSWR-2	Nakahara, City Block, Mirzapur	*O. nivara*
3.	NKSWR-4	Tikara, City Block, Mirzapur	*O. rufipogon*
4.	NKSWR-9	Malua, Patehara, Mirzapur	*O. nivara*
5.	NKSWR-12	Patewar, Madihan, Mirzapur	*O. rufipogon*
6.	NKSWR-16	Dhurkar, Kalwari, Mirzapur	*O. nivara*
7.	NKSWR-25	Godhana, Niyamatbad, Chandauli	*O. nivara*
8.	NKSWR-26	Mahoharpur, Niyamatbad,Chandauli	*O. nivara*
9.	NKSWR-32	Lokmanpur, Saidraja, Chandauli	*O. rufipogon*
10.	NKSWR-34	Amada, Barahani, Chandauli	*O. rufipogon*
11.	NKSWR-35	Baruin, Jamania, Ghazipur	*O. nivara*
12.	NKSWR-36	KaseraPokhara, Jamania, , Ghazipur	*O. nivara*
13.	NKSWR-37	Dildarnagar, Bhadaura, Ghazipur	*O. nivara*
14.	NKSWR-41	Bara, Bhadaura, Ghazipur	*O. nivara*
15.	NKSWR-42	Charitraban, Naibazar, Buxar	*O. rufipogon*
16.	NKSWR-46	Chilkahar, Chilkahar, Ballia	*O. rufipogon*
17.	NKSWR-48	Nakahara, Garwar, Ballia	*O. rufipogon*
18.	NKSWR-49	Hishampur, Bemra Bari, Ballia	*O. rufipogon*
19.	NKSWR-51	Near Suraha Tal, Ballia	*O. rufipogon*
20.	NKSWR-53	Inside SurahatalBallia	*O. rufipogon*
21.	NKSWR-54	Deorara, Bansdih, Ballia	*O. rufipogon*
22.	NKSWR-55	Maniyar, Maniyar, Ballia	*O. rufipogon*
23.	NKSWR-57	Shisotar, Nawanagar, Ballia	*O. rufipogon*
24.	NKSWR-64	Maturi, FatehpurManda, Ballia	*O. rufipogon*
25.	NKSWR-65	Maturi, FatehpurManda, Ballia	*O. rufipogon*
26.	NKSWR-70	Rajapur, Mohammadabad Gohna, Mau	*O. rufipogon*
27.	NKSWR-73	Naretha, Jahanaganj, Azamgarh	*O. nivara*
28.	NKSWR-75	Mehnagar, Mehnagar, Azamgarh	*O. nivara*
29.	NKSWR-82	Shishwara, Martinganj, Azamgarh	*O. rufipogon*
30.	NKSWR-84	Kotila, Rani kiSarai, Azamgarh	*O. nivara*
31.	NKSWR-85	Kotila, Rani kiSarai, Azamgarh	*O. nivara*
32.	NKSWR-86	Shahpur, Jahanaganj, Azamgarh	*O. rufipogon*
33.	NKSWR-97	Lahangpur, Jalalpur, Jaunpur	*O. nivara*
34.	NKSWR-98	TrilochanMahadev, Jalalpur, Jaunpur	*O. rufipogon*
35.	NKSWR-99	BindaMorh, PindraBajar, Varanasi	*O. nivara*

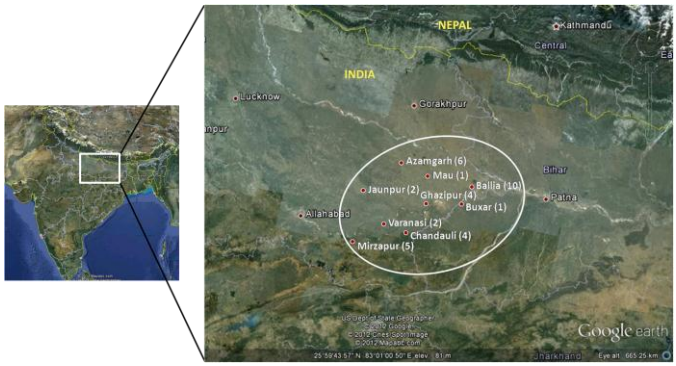

Fig 1. Geographical locations of *O. rufipogon* and *O. nivara* accessions collected from eastern IGP. Figures in parenthesis showed the number of samples analysed from the respective district.

breeding. In the present study, an *O. rufipogon* accession NKSWR-48 showed highest grain yield, spikelet fertility, kernel length, number of filled grain and total grain. Recently, QTL analysis in *O. rufipogon* × *O. sativa* population have suggested that *O. rufipogon* harbour loci which can enhance yield potential in an *O. sativa* background (Marri et al., 2005). Therefore, NKSWR-48 may be used in breeding program for improvement of yield related traits.

Need for conservation actions in eastern IGP

The *O. rufipogon* and *O. nivara* populations of eastern IGP are poorly characterized but are potentially very important gene pool for rice improvement. The widespread distribution of the two species in the eastern IGP (Fig. 1) indicates that these species are secure in the wild but there is great pressure on this habitat due to developmental needs of the growing human population, hence conservation of this gene pool is an urgent requirement. Our limited knowledge of the genetic diversity of this gene pool suggests that the loss of local populations is a real possibility. Risks for the loss of these populations include competition with weeds and land clearing for agriculture and developmental activities. Novel genetic variants and even undescribed species may be present in this region. Conservation of these genetic resources

Table 2. SSR markers, repeat motifs, linkage group, number of alleles and their PIC used in genetic diversity.

SSR	SSR mottif	Linkage group*	No. of alleles	PIC
RM431	(AG)16	1	2	0.79
RM297	(GA)13	1	3	0.88
RM128	(GAA)9	1	2	0.74
RM29	(GA)7	2	2	0.74
RM145	(GA)30	2	2	0.78
RM514	(AC)12	3	2	0.84
RM22	(GA)22	3	3	0.88
RM131	(CT)9	4	2	0.73
RM13	ATTA(GA)6TA(GA)TA(GA)16TTGG	5	2	0.80
RM206	(CT)21	5	3	0.84
RM11	GACA(GA)17GAAA	7	2	0.76
RM433	(AG)13	8	2	0.75
RM25	(GA)18	8	2	0.60
RM152	(GGC)10	8	3	0.87
RM264	(GA)27	8	3	0.89
RM149	(TA)10	8	2	0.87
RM38	(GA)16	8	3	0.90
RM 316	(GT)8-(TG)9(TTTG)4(TG)4	9	3	0.87
RM215	(CT)16	9	2	0.73
RM271	(GA)15	10	2	0.78
RM167	(GA)16	11	2	0.84
RM286	(GA)16	11	3	0.88
RM17	(GA)21	12	2	0.68
RM20	(ATT)14	12	3	0.51
RM210	(CT)23	12	3	0.77
Total			60	
Average			2.4	0.79

***** a *linkage group* is a collection of genes/markers that are close enough in a genome to be inherited together.

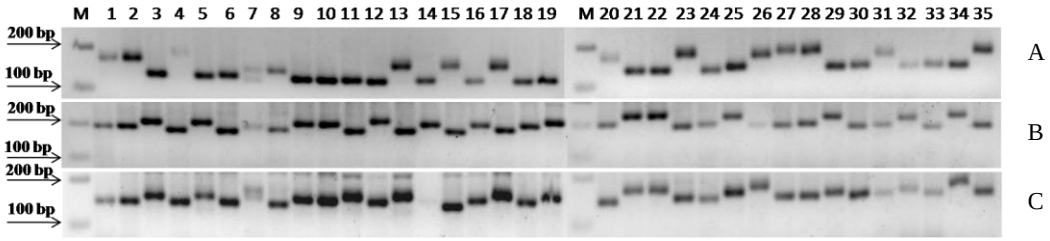

Fig 2. Agarose gel electrophoretic pattern of 35 wild rice accessions generated by using SSR markers RM206 (A), RM152 (B) and RM264 (C), where M is 100 bp DNA size marker and numbers 1-35 represent wild rice accessions as described in Table 1.

requires more extensive exploration, collection of useful germplasm and careful molecular analysis to ensure that this diverse resource remains available to support rice improvement.

Materials and methods

Plant materials

Panicles of 35 different wild rice genotypes were collected from natural habitats of eastern Uttar Pradesh and Buxar district of Bihar, India by Dr. N. K. Singh under the National Professor project at NRCPB, IARI, New Delhi (Table 1; Fig.1). All the accessions were grown at Agricultural Research Farm, Banaras Hindu University, Varanasi, India in a complete randomized block design in single rows in three replications during crop season *Kharif* 2011. The recommended fertilizers doses and cultural practices were followed to raise a good crop.

Field evaluation of morphological traits

The data on 14 morphological and yield related traits were recorded from ten well grown plants of each genotype in each replication. Fourteen traits were assessed, namely, 1) days to 50% flowering, 2) days to maturity, 3) leaf length, 4) leaf width, 5) number of effective tillers per plant, 6) plant height, 7) panicle length, 8) total number of filled grains per panicle, 9) total number of grains per panicle, 10) spikelet fertility, 11) test weight (100 seeds), 12) kernel length, 13) kernel width and 14) yield per plant. The panicles of accessions showing shattering characteristics were observed daily and filled grains were plucked one by one 1-2 days before shattering and stored. Univariate statistical analysis including means, range and coefficient of variation were analyzed.

DNA extraction

Young leaves were collected from two week old plantlets of each genotype grown in the growth chamber. From each accession 40 mg leaves were placed in 1.2 ml collection microtubes (QiagenTissueLyser II, Qiagen, U.S.A.) and in each microtube 3 mm tungsten beads were dispensed by bead dispenser and kept at -80 ^{0}C for 4 hrs. After that tissues were disrupted and homogenized by QiagenTissueLyser to a fine powder at frequency of 30 vibrations/seconds for 30 seconds. Fine powdered leaf samples were then used for isolation of genomic DNA using CTAB method (Doyle & Doyle, 1987). The DNA was quantified spectrophotometrically (Perkin

Table 3. Variation for morpho-physiological traits in thirty five wild rice accessions.

Accessions	DF*	DM	LL	LW	TN	PH	PL	FG	TG	SF%	TW	GY	KL	KB
NKSWR-1	117.33	150.00	68.14	1.42	5.67	161.46	22.09	78.22	103.10	76.00	21.00	20.24	5.83	1.94
NKSWR-2	115.67	148.00	60.24	1.29	5.46	141.32	19.97	49.16	74.42	66.08	21.83	17.26	5.44	2.16
NKSWR-4	114.33	149.67	62.99	1.15	8.19	147.13	17.72	53.83	80.69	66.70	21.00	22.23	6.14	1.75
NKSWR-9	112.67	152.00	60.71	1.27	4.86	154.31	21.77	56.49	86.42	65.47	24.53	20.44	6.15	2.24
NKSWR-12	114.67	151.67	60.65	1.10	6.84	143.75	22.54	46.30	73.81	62.57	21.53	20.96	6.27	1.97
NKSWR-16	114.33	150.33	54.10	1.34	6.15	147.83	24.56	61.74	90.68	68.07	25.33	25.33	5.87	2.38
NKSWR-25	115.33	142.00	62.35	1.29	6.68	144.69	24.27	51.93	94.76	54.97	22.33	22.13	5.54	1.84
NKSWR-26	120.00	154.33	68.14	1.39	7.08	156.05	23.41	36.66	111.67	32.82	14.10	10.99	5.69	2.02
NKSWR-32	115.33	145.00	78.90	1.25	5.62	153.93	22.76	60.81	81.24	74.80	22.87	20.24	6.17	1.99
NKSWR-34	115.33	134.67	63.49	1.11	6.72	151.85	20.00	71.53	98.37	72.83	23.63	25.70	6.22	1.97
NKSWR-35	115.67	144.67	72.82	1.21	7.23	154.28	21.57	76.36	101.03	75.69	23.53	26.69	6.21	1.99
NKSWR-36	114.33	150.33	59.62	1.12	6.41	151.03	20.00	56.84	79.79	71.56	20.60	19.33	6.22	1.95
NKSWR-37	117.33	147.67	67.90	1.57	5.46	150.84	25.27	89.81	137.38	65.40	21.40	25.00	5.70	2.23
NKSWR-41	114.33	149.33	60.52	1.25	7.66	150.80	23.12	62.93	103.91	60.54	23.80	24.99	6.02	2.05
NKSWR-42	112.00	141.33	62.73	1.41	5.81	152.01	20.46	40.85	70.72	57.90	21.87	17.30	5.53	1.96
NKSWR-46	115.67	145.67	71.65	1.04	6.40	128.73	23.21	55.11	77.28	71.50	24.40	24.47	6.23	1.63
NKSWR-48	113.67	155.00	59.78	1.64	2.54	192.20	27.22	231.90	267.29	86.77	31.80	36.99	7.01	2.37
NKSWR-49	114.00	145.33	58.68	1.21	6.38	135.71	22.23	74.35	95.76	77.82	24.80	26.70	6.42	2.07
NKSWR-51	91.00	131.00	56.51	1.78	4.69	186.27	24.36	121.89	176.69	69.01	29.30	31.28	6.24	2.20
NKSWR-53	115.00	156.00	55.37	1.71	3.24	210.83	28.50	142.93	222.43	64.28	19.12	23.46	5.72	1.27
NKSWR-54	115.67	144.00	55.74	1.58	3.85	184.58	23.43	19.13	80.68	23.44	20.47	7.89	6.01	2.32
NKSWR-55	125.33	155.33	62.16	1.33	6.24	140.14	20.30	42.80	72.36	59.14	23.57	20.04	6.55	2.27
NKSWR-57	94.00	128.67	65.64	1.25	5.41	160.27	21.27	40.65	92.89	43.69	25.15	18.71	6.02	2.22
NKSWR-64	114.00	149.33	70.73	1.59	4.76	171.94	27.40	115.36	140.30	82.36	31.53	35.26	6.43	2.51
NKSWR-65	120.67	155.33	76.05	1.16	4.44	139.12	20.40	35.08	67.61	51.69	18.63	11.49	5.02	1.12
NKSWR-70	116.33	149.67	68.81	1.54	3.83	159.27	23.47	73.56	96.60	76.20	27.03	24.08	6.02	2.45
NKSWR-73	114.67	151.00	59.00	1.28	5.63	148.63	21.36	74.97	124.79	60.10	21.47	27.01	5.66	2.08
NKSWR-75	116.33	149.00	64.56	1.68	3.54	153.54	27.16	117.41	167.57	70.14	33.50	35.36	6.42	2.51
NKSWR-82	117.00	146.67	72.50	1.32	5.95	137.82	23.56	32.62	54.61	59.80	21.74	16.91	6.10	1.56
NKSWR-84	104.33	142.67	58.37	1.34	5.64	158.99	24.28	62.60	106.48	58.77	23.17	25.81	6.08	1.62
NKSWR-85	106.33	145.33	52.62	1.40	5.54	143.14	22.14	52.31	90.01	58.30	21.53	23.42	5.75	1.96
NKSWR-86	111.67	137.67	65.77	1.23	4.60	133.84	25.36	53.22	70.18	75.94	22.53	19.90	6.12	2.22
NKSWR-97	113.00	142.33	64.00	1.64	4.88	149.08	22.61	58.72	112.64	52.11	24.30	22.61	6.31	1.93
NKSWR-98	115.33	156.67	60.69	1.80	4.32	184.05	26.03	78.09	139.08	56.17	28.93	31.68	6.62	2.63
NKSWR-99	117.00	157.00	55.58	1.30	6.28	154.79	24.54	61.79	113.05	54.63	21.53	24.59	6.19	2.05
Mean	113.7	147.28	63.36	1.37	5.54	155.26	23.1	69.66	107.32	63.52	23.54	23.04	6.05	2.04
Max	125.33	157	78.9	1.8	8.19	210.83	28.5	231.9	267.29	86.77	33.5	36.99	7.01	2.63
Min	91	128.67	52.62	1.04	2.54	128.73	17.7	19.1	54.61	23.44	14.1	7.89	5.02	1.12
SD (±)	6.35	6.88	6.35	0.21	1.27	17.73	2.44	38.77	44.29	12.98	3.88	6.43	0.37	0.32
CV %	26.4	34.2	54	53	95	30.4	82.8	86.9	94.6	39.1	26.7	71.6	16.7	26

*DF = days to 50% flowering; DM = days to maturity; LL = leaf length (cm); LW = leaf width (cm); TN = total number of tillers; PH = plant height (cm); PL = panicle length (cm); FG = total number of filled grain per panicle; TG = Total number of grains per panicle; SF% = spikelet fertility; TW = test weight (g); GY = grain yield per plant (g); KL = kernel length (mm); KB = kernel bredth (mm)

Elmer, Singapore) by measuring A_{260}/A_{280} and DNA quality was checked by electrophoresis in 0.8% agarose gel.

SSR analysis

A total of 80 SSR markers, synthesized by Eurofins Genomics (Bangalore, India), were initially evaluated using four most morphologically diverse genotypes for their capability to amplify clear, reproducible and polymorphic DNA bands. Later, 25 polymorphic markers were selected for the analysis of all the 35 genotypes (table 2). The amplification was carried out in 15µl of reaction mixture containing 30 ng genomic DNA, 1.5 mM PCR buffer (MBI Fermentas, USA), 400 µM dNTPs (MBI Fermentas), 1 U Taq DNA polymerase (MBI Fermentas) and 0.4 µM primer using a thermal cycler (Mastercycler gradient, Eppendorf). Thermal cycling program involved an initial denaturation at 94^0C for 4 min, followed by 34 cycles of denaturation at 94 ^{0}C for 45 sec, annealing at 2 °C below Tm of respective primers for 30 sec, primer extension at 72 ^{0}C for 30 sec, followed by a final extension at 72 ^{0}C for 8 min. The amplified PCR products along with a 100 bp DNA marker ladder (MBI Fermentas) were size fractioned by electrophoresis in 2.5% agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/ml) in a gel documentation system (BIO-RAD, USA).

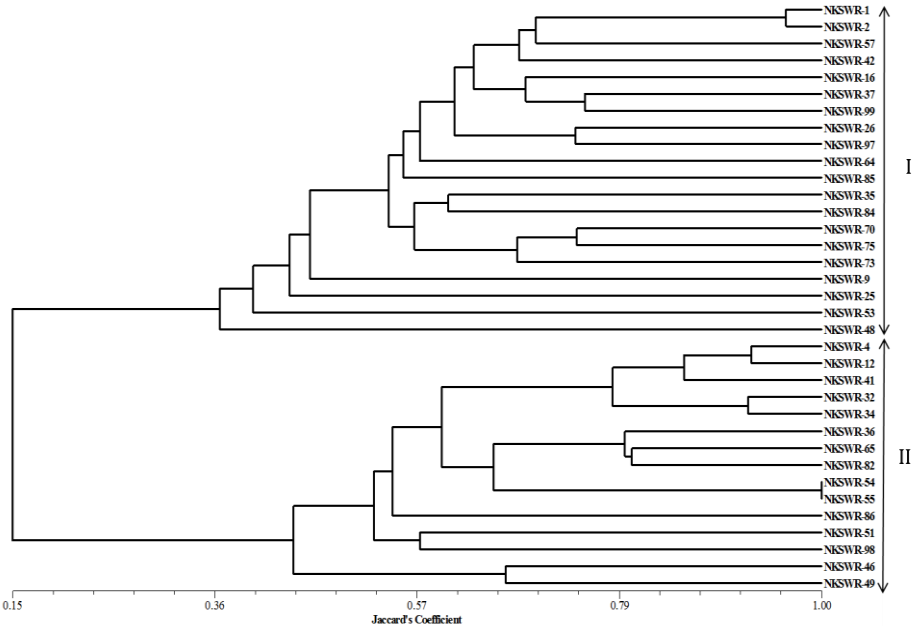

Fig 3. UPGMA dendrogram based on Jaccard's similarity coefficient showed the clustering of 35 wild rice accessions in two broad groups corresponding to *O. nivara* (cluster I) and *O. rufipogon* (cluster II).

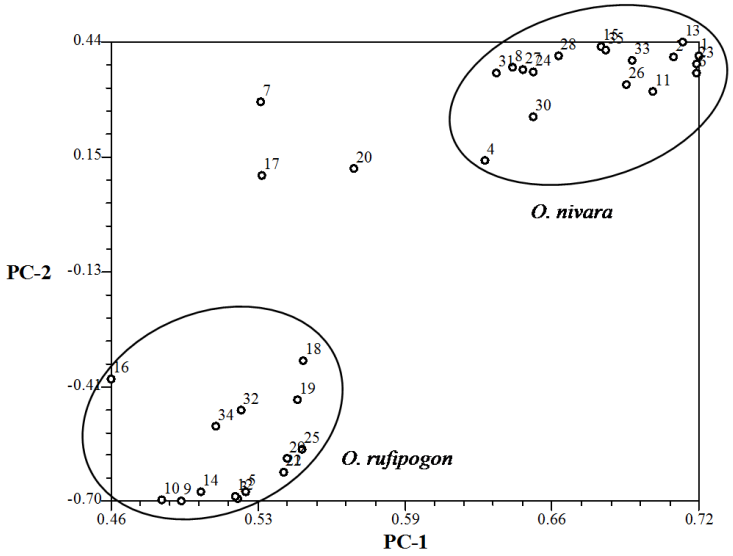

Fig 4. Patterns of relationship among 35 wild rice accessions revealed by principal component analysis based on 25 SSRs. Numbers 1-35 represents different wild rice accessions. The two groups as showed by circles broadly correspond to *O. nivara* and *O. rufipogon*.

Fig 5. Variability in grain colour, size and shape of 35 wild rice germplasm lines, the serial number represents wild rice accessions as described in Table 1.

Data analysis

The SSR bands were scored manually for the presence (1) or absence (0) across all the genotypes. Only reproducible and clearly distinguished bands were taken into consideration. Polymorphic information content (PIC), a measure of polymorphism at a marker locus, was calculated according to Anderson et al. (1993):

$$\text{PIC} = 1 - \sum_{i=1}^{K} P_i^2$$

where P_i is the frequency of the *i*th allele and *k* is the total number of different alleles at the specific locus. Cluster analysis was performed using NTSYSpc v 2.1 software (Rohlf, 1998). Pair-wise combinations of genotypes were employed to calculate Jaccard's similarity coefficient (GS) = a/(n - d), where a is the number of positive matches, n is the total sample size, and d is the number of negative matches (Jaccard, 1908). This matrix was subjected to cluster analysis by the unweighted pair-group method (UPGMA; Sneath & Sokal, 1973) and the dendrogram was constructed using the SAHN module of NTSYSpc software package. The binary data was also subjected to principal component analysis (PCA) using the EIGEN and PROJ modules of NTSYS Pc.

Conclusion

The *O. rufipogon* and *O. nivara* populations are potentially very important gene pool for rice improvement. The distribution of these species in the eastern Indo –Gangetic plains of India indicated the existence of rich genetic diversity and conservation of this gene pool is required. It would be worthwhile to commission a regional wild rice germplasm exploration. Further, a thorough molecular analysis will ensure use of diverse wild rice accessions to aid in cultivated rice improvement program.

Acknowledgement

Authors are thankful to Indian Council of Agricultural Research for the National Professor (B. P. Pal Chair) project on wild rice to NKS and to Department of Biotechnology, Government of India for financial support (Grant no. 07/473). Invaluable help rendered by Sri Vivek Singh and Sri O. P. Singh during the collection of wild rice samples from the remote villages is gratefully acknowledged.

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