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Assessment of genetic diversity in medicinal rices using microsatellite markers

L. Behera[1*], B.C. Patra [1], R.K. Sahu [1], A. Nanda [1], S.C. Sahu [1], A. Patnaik [1], G.J.N. Rao[1], O.N. Singh

[1]Central Rice Research Institute, Cuttack-753 006, Odisha, India

***Corresponding author: l_behera@yahoo.com**

Abstract

A set of 36 microsatellite markers distributed over 12 chromosomes of rice were used to assess genetic diversity in 33 medicinal rice genotypes. A total of 169 alleles were amplified, of which 166 were polymorphic. The number of alleles detected per locus ranged from 2 to 9 with an average of 4.69 alleles per locus. The polymorphism information content (PIC) ranged between 0.24 and 0.956 with an average of 0.811 per locus, indicating the suitability of the microsatellite markers for detecting genetic diversity among these rice genotypes. All the rice genotypes showed the presence of multiple alleles. Genetic similarities among genotypes varied from 0.239 to 0.827 with an average of 0.5. The cluster analysis grouped all the genotypes into two major clusters at 0.43 level of genetic similarity. The first three principal coordinates explained more than 0.63 of total genetic variation. All the genotypes included in the study could be uniquely distinguished from each other. The data provides basic information on medicinal rice genotypes of India which will be useful for future reference and to protect these unique rices under Intellectual Property Rights (IPR) regime.

Keywords: DNA fingerprinting; genetic diversity; medicinal rice; microsatellite marker; molecular marker.
Abbreviations: AFLP-Amplified Fragment Length Polymorphism, CTAB- Cetyl Trimethyl Ammonium Bromide, PCA-Principal Component Analysis, SSR- Simple Sequence Repeat.

Introduction

Rice is the staple food of more than 50% of the world's population. Among the rice growing countries in the world, India has the largest area under rice crop and ranks second in production next to China. The cultivated rice (*Oryza sativa* L.) is rich in genetic diversity apart from highly diverse 21 wild progenitors and the African cultivated rice, *Oryza glaberrima* Steud. In addition to staple food, rice has extensive protective and curative properties against human ailments like epilepsy, chronic headache, rheumatism, paralysis, skin diseases, diabetes, arthritis, indigestion, blood pressure, colon cancer, internal rejuvenation of tissues and overcoming postnatal weaknesses (Kirtikar and Basu, 1935). Medicinal uses of rice are well documented in Ayurvedic system of medicine in the ancient Indian Ayurvedic books viz., Susrutha Samhita, Charak Samhita and Astanga Hridayam. Medicinal rices appear to be confined to specific pockets of Central India, Southern India (Kerala, Tamil Nadu and Karnataka) and North eastern hilly regions. Most of these varieties are traditionally cultivated in small scale and in restricted pockets. Rice surveys in Chhattisgarh have led to the identification of many traditional rice varieties like Alcha, Baissor, Gathuhan, Laicha, Bhejri, Karhani and Kalimooch possessing medicinal properties (Das and Oudhia, 2001). The variety, Alcha is used for treatment of pimples while Baissor is used for chronic headache. The traditional varieties, Alcha and Kuzhiyadichan (Kulikulichan) in Tamil Nadu (Arumugasamy et al., 2001), Karibhatta and Atikaya in Karnataka (Balchandran et al., 2004), and Njavara (Navara), Chennellu, Erumakkari, Raktasali and Kunjinellu in Kerala (Sujith Kumar, 1999) are used for treatment of various diseases. The Njavara is the most widely used medicinal rice in the world. This rice can also be prompted as a health food. The promotion of medicinal rices can open up greater awareness, benefit a broader public and result in increased income for the poor farming community of India. Unambiguous, reliable, fast and cost-effective identification, assessment of genetic diversity and relationships within and among crop species and their wild relatives is essential for the effective utilization and protection of plant genetic resources (Paterson et al., 1991; Barcaccia, 2009). Traditionally used morphological and biochemical markers are not discriminative enough, warranting more precise techniques. Further, these markers are not reliable because many characters of interest have low heritability and genetically complex in nature. Molecular marker technology provides powerful tool for assessment of genetic diversity among cultivars, identification of cultivars and thus add to management and protection of plant genetic resources (Virk et al., 2000). Of the wide array of DNA markers available, microsatellite or simple sequence repeat (SSR) markers are considered to be appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith et al., 1996; Varshney et al., 2005; Ijaz,

2011). Microsatellite markers have been ideal for identification and purity checking of rice varieties (Nandakumar et al., 2004; Singh et al., 2004), characterization of genetic diversity in cultivated (Nagaraju et al., 2002; Ni et al., 2002; Yu et al., 2003; Jain et al., 2004; Kibria et al., 2009; Zhang et al., 2009) and wild rices (Ren et al., 2003; Juneja et al., 2006) and also more distantly related grass species (Ishii and McCouch, 2000). A random set of microsatellite markers should facilitate an unbiased assay of genetic diversity and unambiguous molecular description of rice genotypes. The medicinal properties of different rice cultivars are yet to be supported by sound scientific data. No serious efforts have been made at national level for genetic improvement except for reports on collection, characterization and evaluation of the medicinal rice, Njavara (Menon and Potty, 1998; Sreejayan et al., 2003; Leena Kumary, 2004, Thomas et al., 2006; Deepa et al., 2008). The valuable genetic wealth of medicinal rices is being eroded because of their poor yield and introduction of high yielding varieties. Thus, it is highly essential that these valuable rice germplasm are to be collected, conserved, properly characterized, genetically enhanced and documented in view of their importance in IPR regime. The present study was undertaken to assess the extent of genetic diversity present among the medicinal rice genotypes using microsatellite markers.

Results and discussion

Allelic diversity of microsatellite markers

All the 33 microsatellite primers revealed polymorphism between genotypes. A total of 169 bands/alleles were amplified, of which 166 (98.22%) were polymorphic. The number of alleles per locus ranged from 2 to 9 with an average of 4.69 (Supplementary Table 1). Two loci, RM 203 and RM333 amplified highest number alleles (9 in each case). The lowest number of alleles was observed in RM432 loci. The number of alleles detected in the present study corresponded well with earlier reports (Nagaraju et al., 2002; Jain et al., 2006; Juneja et al., 2006; Ram et al., 2007). However, Jain et al. (2004) obtained higher number of alleles (3 to 22) as compared to present study, because of inclusion of Basmati as well as non-Basmati varieties and the use of fluorescent techniques in their study. The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and materials used in the studies (Ni et al., 2002; Blair et al., 2002; Lu et al., 2005; Jayamani et al., 2007; Thompson et al., 2009; Kaushik et al., 2011). Microsatellite markers with simple tri-nucleotide repeat motifs tended to detect a greater number of alleles (average: 5.71, n=7) than those with di-nucleotide repeat motifs (average: 4.48, n=21) (Supplementary Table 2). Non significant correlation ($r = 0.079$, $P > 0.10$) was found between number of alleles amplified per locus and number of repeats in simple motif of a SSR locus (Supplementary Table 4). Similar to our observation, Nagaraju et al. (2002) and Juneja et al. (2006) found no direct correlation between number of repeats and number of alleles detected in aromatic and wild rice materials, respectively. However, Ni et al. (2002) and Yu et al. (2003) found positive correlation between number of alleles amplified and number of repeats within a microsatellite marker. Cho et al. (2000) and Jain et al. (2004) observed that SSR loci with di-nucleotide repeats detected greater number of alleles than those with tri-nucleotide repeats. Kaushik et al. (2011) found SSR loci with complex mixed repeats detected highest number of alleles followed by those with simple tri-nucleotide, di-nucleotide and tetra-nucleotide repeat motifs. Eleven unique alleles (6.51%) were observed at 8 SSR loci. An allele that was observed only in one of the 36 rice genotypes was considered to be a unique allele. Unique alleles are important because they may be diagnostic of a particular genotype and useful for breeding purpose. Number of unique alleles per locus varied from 1 to 2 (RM 307, RM 333 and RM 470) (Table 1). All the markers amplifying unique alleles showed high level polymorphism information content (PIC) values (> 0.75) (Supplementary Table 1).Three medicinal rice genotypes, Ratanchudi, Gudamatia and Kandul which are used as tonic for general weakness, treatment of diabetes and paralysis, respectively amplified two unique alleles each (Table 1). Two high yielding varieties (Kalinga III and Swarna) and three medicinal genotypes (Bangali, Kalamali and Gotia) amplified an unique allele each. The occurrence relatively higher number of unique alleles in the medicinal rice genotypes indicates its potentiality as a reservoir of novel alleles for crop improvement. Saini et al. (2004) identified 58 unique alleles (36.2%) among Basmati and non-Basmati rice varieties. These unique alleles were observed at 25 of 30 SSR loci. Davierwala et al. (2000) identified many alleles specific to elite cultivars of India using microsatellite markers. Similarly, others also detected unique alleles both in cultivated and wild rices (Giarrocco et al., 2007; Wong et al., 2009). Sixty eight (40.24%) high frequency and 90 (53.25%) of low frequency alleles were identified among 36 medicinal rice genotypes. On an average, 56.9% of the 36 rice genotypes produce any low frequency alleles. The presence of high proportion of low frequency alleles in medicinal rice genotypes indicated that they make a greater contribution to overall genetic diversity of the collection. Jain et al. (2004) observed that 53.6% of 69 rice genotypes shared common alleles at any locus. Thompson et al. (2009) indicated that on an average, 62% of the 190 rice accessions of Indonesia shared a common major allele at any given of SSR locus. Similar results were also observed by others (Saini et al., 2004; Lu et al., 2005; Jayamani et al., 2007). All the rice genotypes including high yielding varieties, Kalinga III, Vandana and Swarna showed the presence of multiple alleles. Thirty four of 36 microsatellite loci amplified one to five multiple alleles per locus (Supplementary Table 1). For any given marker, multiple alleles were detected in an average of 37.42% genotypes per locus, indicating presence of high level of heterogeneity in the genotypes. Number of genotypes with multiple alleles vary from one (2.78 %) at RM 30 locus to 36 (100%) at RM 20, RM 189 and RM269 loci (Supplementary Table 1). Figure 1 shows the amplification of multiple alleles in all the genotypes by RM 20 locus. A positive correlation ($r = 0.665$, $P < 0.0001$) was found between total number of alleles and number of multiple alleles amplified per SSR locus (Supplementary Table 4). Rice is a self pollinated, diploid crop species. The

microsatellites usually reveal single copy, homozygous loci and allelic heterogeneity is rare in respect of pure line varieties. Hence, the presence of multiple alleles in cultivars is generally an indication of seed mixtures, mixture of pure lines or residual heterozygosity (Jain et al., 2004). This phenomenon is quite common in landraces that contribute to their broad genetic plasticity to adapt themselves to different agro-climatic conditions in traditional farming systems (Olufowate et al., 1997). In the present study, the heterozygosity could not be differentiated from heterogeneity because the DNA samples were extracted from bulked leaf samples. Analysis of 43 Australian cultivars with microsatellite markers indicated that 42% of cultivars were heterogeneous (Garland et al., 1999). They observed the high level of heterogeneity at microsatellite locus, OSR6. Jain et al. (2004) observed multiple alleles in 62% of Indian aromatic and Basmati rice accessions and even in modern varieties such as IR 36 and Nipponbare. Similar results were also obtained both in cultivated and wild rices (Yu et al., 2003; Saini et al., 2004; Lu et al., 2005; Jain et al., 2006; Juneja et al., 2006; Giarrocco et al., 2007; Jayamani et al., 2007).

Polymorphism information content

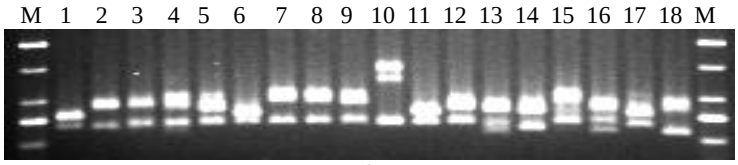
The Polymorphism information content **(**PIC) value provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. The PIC values for 36 SSR loci in our study varied from 0.24 (RM 189) to 0.956 (RM 333) with an average of 0.811 (Supplementary Table 1). All the loci except the three (RM 259, RM 189 and RM 269) showed high PIC values (>0.60). The estimated average PIC values are relatively higher than the average PIC values as reported by others (Lu et al., 2005; Juneja et al., 2006; Joshi et al., 2010) and thus might be due to higher genetic diversity present in selected medicinal rice genotypes. Moreover, the SSR markers used in the study were selected on the basis of their high PIC values reported earlier. Higher PIC values for some SSRs similar to our findings were also reported in the literature (Garland et al., 1999; Giarrocco et al., 2003; Juneja et al., 2006; Jayamani et al., 2007; Ram et al., 2007). Microsatellite markers with simple tri-nucleotide repeat motifs detected higher polymorphism (Mean: 0.853; n = 7) than those with di-nucleotide repeat motifs (Mean: 0.788 n = 21) (Supplementary Table 2). No correlation ($r = 0.147$, $P > 0.10$) was observed between number of repeats of simple motif and PIC value of a SSR locus. However, a positive correlation ($r = 0.4$, $P < 0.01$) was evident between number of alleles amplified and PIC values (Supplementary Table 4). Jain et al. (2006) had also observed that PIC values showed a positive correlation with total number of alleles at SSR locus ($P = 0.01$). Microsatellite loci with (CTT)n and AT-rich di- and tri-nucleotide repeat motifs amplified a greater number of alleles and revealed greater polymorphism(Supplementary Table 3). Two of the microsatellite loci, RM 203 and RM 333 having (AT)n and (TAT)n(CTT)n repeats, respectively amplified nine alleles each and had PIC values of 0.914 and 0.956, respectively (Supplementary Table 3). Similar results are also reported by Temnykh et al. (2000) who revealed that (CTT)n and AT-rich tri-nucleotide repeats amplified with higher efficiency and revealed greater polymorphism. Juneja et al. (2006) indicated that the markers, RM 340 and RM 333 having (CTT)n repeat yielded 6-7 alleles and were most informative with PIC value of 0.8.

Genetic diversity and relationship among medicinal rice genotypes

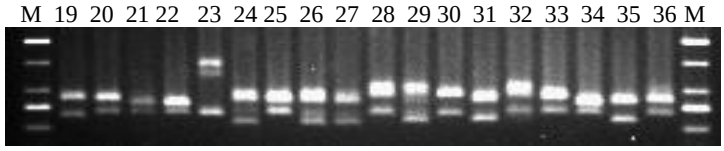
Genetic similarity coefficients of pair-wise comparisons estimated on the basis of all the 36 microsatellite loci ranged from 0.239 to 0.827 with an average of 0.5, indicating a wide range of genetic variation present in the medicinal rice genotypes. The genotype Gadaguta showed the highest similarity with Gudamatia (i.e. 0.827) while Meher showed the least similarity with Kande (i.e. 0.239). High level of similarity was found among Bogasoli and Ratanchudi (0.824) as well as between Bedro and Mancha (0.808). Similar to our observations, other studies using SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice (Ren et al., 2003; Juneja et al., 2006; Jayamani et al., 2007; Joshi et al., 2010). Juneja et al. (2006) analyzed genetic variation among 127 *O. nivara* accessions and two cultivars using 33 SSR markers. Genetic similarities varied from 0.22 to 0.9, indicating a wide range of variability present in wild germplasm. Jayamani et al. (2007) detected a significant genetic variation among the 176 rice accessions originating from 19 countries in the Portuguese working germplasm collection and two standard rice varieties, IR36 and Nipponbare with a genetic similarity coefficient varying between 0.09 and 1.00 using 24 SSR loci. Joshi et al. (2010) analyzed 21 low land and 3 shallow low land rice using 45 SSR markers. Genetic similarity varied from 0.041-0.728. Cluster analysis based on genetic similarity values provided a clear resolution of relationships among all the 36 rice genotypes. The Cophenetic correlation coefficients ($r = 0.803$) revealed the reliability and stability of clustering. Two major clusters were observed at 0.43 of genetic similarity coefficient index (Fig. 2). First major cluster contained 27 medicinal rice genotypes with an average similarity index of 0.568. Further, it was sub-grouped into two clusters, IA and IB having 22 and 5 genotypes, respectively. The similarity coefficients between any two rice genotypes in the sub-cluster, IA ranged from 0.396 (Sindursingha and Chinabhuchi) to 0.827 (Gadaguta and Gudamatia) with an average of 0.574. Second sub cluster, IB was less diverse than first sub-cluster, IA. Interestingly, all the 3 high yielding varieties were included in this sub-cluster along with two medicinal rice genotypes, Kande and Sambalpuri. The similarity coefficients between any two genotypes varied from 0.429 (Kalinga III and Sambalpuri) to 0.626 (Sambalpuri and Vandana) with an average of 0.540. The second major cluster included 9 medicinal rice genotypes with similarity indices from 0.457 (Marangdhan and Mehera) to 0.785 (Limchudi and Katak) with an average of 0.608. The classification of rice genotypes based on 36 SSR primers

Table 1. Microsatellite loci that amplified unique alleles in different rice genotypes.

Microsatellite locus (RM)	Unique allele (bp)	Name of rice genotype
30	100	Bangali
225	260	Ratanchudi
253	100	Gudamatia
307	70	Gudamatia
	190	Kalinga III
335	70	Kalamali
333	310	Swarna
	750	Gotia
428	260	Ratanchudi
470	175	Kandul
	600	Kandul
Total	11	



A



B

Fig 1. DNA profiles of 33 medicinal and 3 high yielding rice genotypes (A and B) obtained with microsatellite locus, RM 20. The lane number corresponds to a rice genotype as given in the Table1, M-50bp DNA ladder. All the rice genotypes show the presence of multiple alleles.

was highly similar with that based on fifteen and ten of the most informative primers with matrix correlation (r) of (+) 0.80 and (+) 0.68, respectively. Thomas et al. (2006) analyzed the genetic diversity of different collections of medicinal rice, Njavara using morphological and molecular (AFLP and SSR) markers. Six groups in Njavara germplasm were identified that could be clubbed under four distinct varietal types. The PCA is one of the multivariate approaches of grouping based on similarity coefficients or variance-covariance values. It is expected to be more informative about differentiation of major groups. The groupings identified by PCA (Fig. 3A, B) were comparable to those identified by UPGMA cluster analysis. More than 0.63 of genetic variation was explained by the first three coordinates, indicating suitability microsatellite markers for genetic clustering. The first, second and third principal coordinates explained 0.517, 0.07 and 0.043 , respectively of total genetic variation in SSR data. Two major clusters were revealed by both two (Fig. 3A) and three principal components (Fig. 3B). Unlike UPGMA analysis, where all genotypes were included in a group, KalingaIII (No.19) and Meher (No. 2) were out of the major clusters, possibly indicating genetic differentiation. The first major cluster included 26 genotypes while second major cluster had 8 genotypes. Jayamani et al. (2007) obtained comparable groupings of Portuguese rice accessions by PCA and UPGMA cluster analysis with some deviations. The groupings identified by PCA were very similar to those identified by the UPGMA tree cluster analysis of the 52 Indian aromatic/ quality rice genotypes (Jain et al., 2004). Aggarwal et al. (2002) observed six clusters in PCA while 3 clusters in UPGMA analysis by characterizing Indian Basmati and other elite genotypes using AFLP markers.

Differentiation of medicinal rice genotypes

A number of microsatellite markers were identified that distinguished between different medicinal and high yielding rice genotypes. All the rice genotypes used in the present study could be distinguished precisely from each other at the level of 18 to 68 polymorphic alleles between individuals in pair wise comparison. However, none of the microsatellite loci could differentiate all the genotypes. The discriminating power of microsatellite loci vary from 0.243 (RM 189) to 0.952(RM 169) with an average of 0.812 (Supplementary Table 1). On the basis of discriminating power, the minimum number of microsatellite loci required to differentiate between genotypes in the present study was found to be three (i.e. RM 169, RM 335 and RM 333). These three SSR loci amplified a total of 23 alleles, all being polymorphic. The frequency of these alleles ranged from 1/36 to 17/36. Similar results were obtained by others

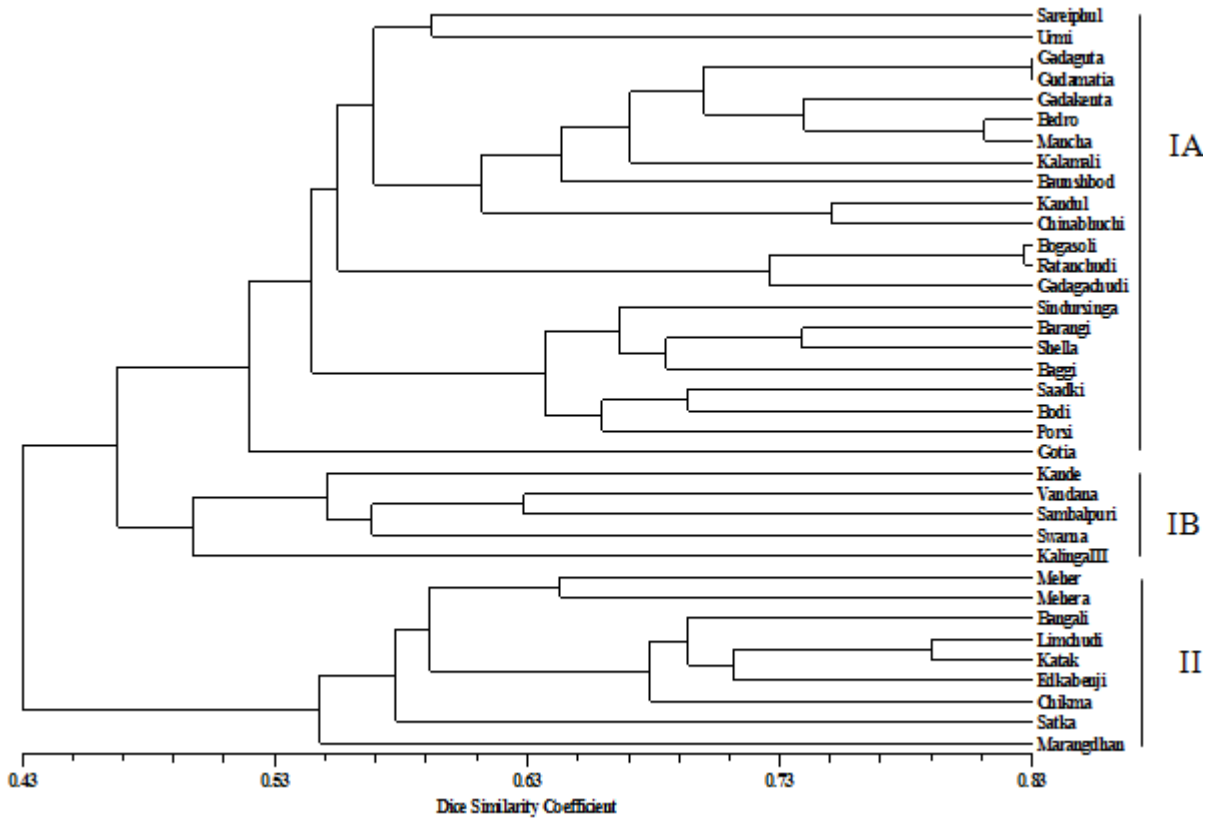


Fig 2. UPGMA dendrogram showing genetic relationship among 33 medicinal and 3 high yielding rice genotypes based on Dice similarity matrix derived from 169 alleles at 36 microsatellite loci. The major clusters and sub-clusters are indicated on right margin.

(Olufowote et al., 1997; Saini et al., 2004). Six well chosen SSLPs were found to be sufficient to discriminate between 71 related lines of rice (Olufowote et al., 1997). Saini et al. (2004) evaluated the genetic diversity among the 18 rice genotypes representatives of the traditional Basmati, cross-bred Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. The minimum number of markers needed to distinguish between all the cultivars was one for AFLP, two for ISSR and five for SSR. Some researchers were able to unambiguously identify and discriminate twenty eight rice varieties which included thirteen high yielding, fourteen local and a wild rice cultivars using only three microsatellite markers. The combination of all the polymorphic and non-polymorphic alleles obtained with all the 36 SSR loci enabled development of DNA profile data set for 33 medicinal rice genotypes along with three high yielding rice varieties as check (data not shown), which can serve as a guide for easy visual comparison of any additional genotype as and when become available.

Materials and methods

Plant materials

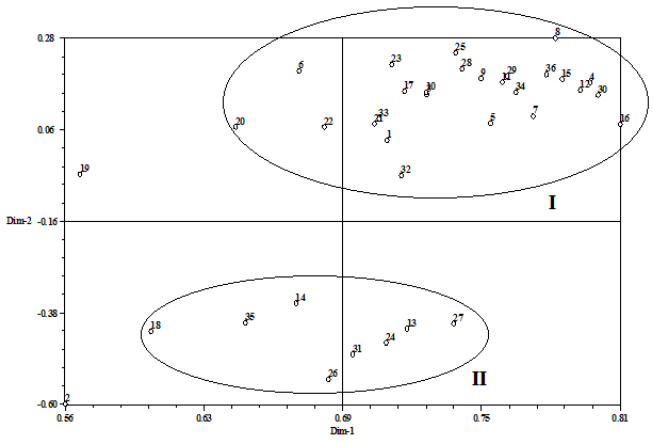
The experimental materials comprised of a set of 33 landraces having different therapeutic values and a set of three high yielding rice varieties as check (Supplementary Table 5). Based on the earlier reports of medicinal rices from Bastar region of Chhattisgarh, India (Das and Oudhia, 2001), these 33 medicinal rice genotypes were collected from farmer's field. During collection, interactions were made with the farmers to record the medicinal uses of rice genotypes along with the prescribed passport data.

Genomic DNA isolation

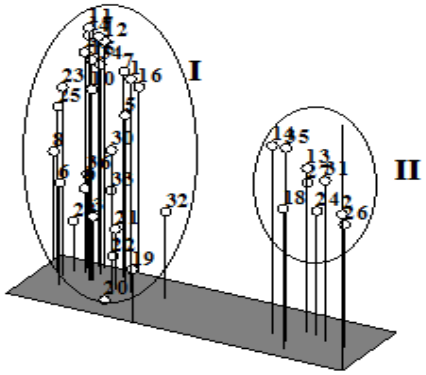
Twenty seeds per genotype were germinated in a Biological Oxygen Demand (BOD) incubator under aseptic condition at 30^0C. Then young seedlings (five days after germination) were transplanted in individual pots in green house. After 20 days of transplanting, young leaves were harvested from 10-15 plants and bulked for each genotype. Genomic DNA was isolated from 3-4 gm of bulked leaf samples following CTAB method (Murray and Thompson, 1990). The quantity was estimated by spectrometrically and by ethidium bromide staining after agarose gel electrophoresis using known concentration of Lambda DNA. The samples were diluted in $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to get final concentration of 15 ng/ µl for amplification.

PCR amplification and electrophoresis

A set of 36 microsatellite markers distributed over 12 chromosomes of rice were used for DNA profiling



(A)



(B)

Fig 3. Two-dimensional (A) and three-dimensional (B) plot of 33 medicinal and 3 high yielding rice genotypes resulting from principal component analysis (PCA) based on the 169 alleles amplified by 36 microsatellite loci. The numbers plotted represent individual rice genotypes that are listed in Supplementary Table 5. Circles in the figures A and B indicate the major clusters. The first three coordinates explained more than 63% of total genetic variability.

(Supplementary Table 1). The primer sequences for these markers can be found in the Gramene website (http://www.gramene.org). The amplification was carried out in a 20 µl reaction mixture volume containing 30 ng of genomic DNA, 1X PCR buffer {75 mM Tris-HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2SO_4$}, 200 µM dNTP mix (MBI Fermantas, Lithuania, USA), 4 picomole of each of forward and reverse primers, 2 mM of $MgCl_2$ and 1U of Taq *(Thermus aquaticus)* DNA polymerase (Biotools, Spain). The PCR was performed in a thermal cycler (Thermal Cycler, Perkin Elmer, Cetus) as per following cycling parameters: initial denaturation at 94^0C for 3 min followed by 36 cycles of denaturation at 94^0C for 1 min, annealing at 55-67^0C (depending upon primer) for 1 min and extension at 72^0C for 1.5 min and final extension at 72^0C for 5 min. The amplified products were separated on 2.5% agarose gel containing ethidium bromide (0.5 µg/ml) using 1X TBE buffer. The gels were visualized under UV radiation and photographed using a gel documentation system (Fluor Chem^{TM} 5500, Alpha Innotech, USA) to detect polymorphism. The size of amplified bands was determined by using size standards (50 bp DNA ladder, MBI Fermentas, Lithuania).

Data analysis

The amplified bands/alleles were scored as present (1) or absent (0) for each genotype and primer combination. The data were entered into a binary matrix and subsequently analyzed using the computer package, NTSYS-pc (Version 2.02)(Rolf, 1998). The number of high frequency alleles per locus (HFA), number of low frequency alleles (LFA), number of multiple alleles (MA), number of polymorphic alleles (PA), polymorphism information content (PIC), total number alleles (TA) and number of unique alleles (UA) were calculated to assess diversity of alleles of marker locus. An allele that was observed in > 30% of the 36 rice genotypes was considered to be a high frequency/ abundant/ common allele while an allele having frequency between 5% and 30% is called as a low frequency/ intermediate allele. The polymorphism information content (PIC) was calculated using the formula, PIC = 1-$\sum Pij^2$, where Pij is the frequency of i^{th} allele for the j^{th} locus and summation extends over n alleles (Anderson et al., 1993). In order to find the efficiency of SSR markers for differentiation of genotypes, the discriminating power (D) of each marker loci was calculated following formula, Dj = 1-Cj = 1-$\sum$ Pi (NPi-1)/(N-2), where Dj is discriminating power of j^{th} locus, Pi is frequency of i^{th} allele, Cj confusion probability of j^{th} locus (Tessier et al., 1999). Further, in order to know minimum number of marker loci required to identify and differentiate genotypes from each other, total number of non-differentiated pairs(Xj) of genotypes were calculated for the j^{th} locus using formula, Xj = {N(N-1)/2}Cj. Dice genetic similarity coefficients were calculated and used to assess the genetic relationship among 48 low land rice genotypes (Nie and Li,1979) and then were used to construct dendrogram using unweighted pair group method using arithmetic averages (UPGMA) and sequential agglomerative hierarchal nested (SHAN) cluster analysis. The Cophenetic correlation coefficient (Lapointe and Legendre, 1992) was calculated to measure the goodness of fit of clusters. Principal component analysis (PCA) was performed to high light the resolving power of the ordination.

Conclusion

The present study clearly indicated that microsatellite markers are useful in assessing genetic diversity in medicinal rice genotypes. All the genotypes analyzed could be distinguished from each other. A basic molecular data set was created for the medicinal rice genotypes which can be used for variety registration, preventing misappropriation and protecting the plant breeders as well as farmers' rights. We suggest a wider survey and collection of medicinal rice genotypes from different geographical regions of India in order to conserve maximum diversity, and utilize this unique and valuable

resource in breeding programs benefit of both farmers and consumers.

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