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Full Length Research Paper

DNA fingerprinting and estimation of genetic diversity among hybrid rice parental lines (*Oryza sativa* L.) using simple sequence repeats (SSR) markers

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Molecular fingerprinting and genetic distance analysis of twelve commercial hybrid rice parental lines were carried out using simple sequence repeats (SSR) markers. Out of hundred SSR markers screened, sixty two were found to be polymorphic. A total of 203 alleles were recorded as 0 to 1 data set (presence-1 and absence-0) with an average of 3.2 alleles per loci. Most of the primer sets generated fingerprint markers for rice lines used in this study which would be useful in their hybrid detection with an example. Commercial rice hybrid KRH-2 can be detected using its male parent KMR-3 specific markers RM297, RM442, RM541, RM584 and RM107 and female parent IR58025A specific markers RM529, RM489, RM589, RM533 and RM182 identified in this study. NTSYS cluster analysis of genotypic data set distinguished maintainer and restorer groups into two different clusters with the mean genetic distance value of about 22%. The results indicated the low level of genetic diversity exists among the commercial hybrid rice parental lines, suggesting the need to broaden the genetic base in order to maximize heterosis in hybrid rice breeding program.

Key words: Cultivar identification, genetic diversity, hybrid rice, simple sequence repeats (SSR) markers.

INTRODUCTION

Rice provides 60 to 70% energy intake for almost half of the world population. It is produced globally on more than 150 million hectares with an annual production of about 685 million tons. In India, it occupies about one-quarter of cropped area and contributes 40 to 43% of total food grain production and 46% of total cereal production (Krishnaih and Shoba Rani, 2000). However, the growing domestic population of 1.3% per year urges to increase the rice productivity of double the quantity from the current status of 133 million tons. Therefore, strategies such as exploitation of hybrid vigor, improvement of plant type, identification and utilization of yield-enhancing genetic sources in the breeding program etc are being explored. Recent breakthroughs in tropical hybrid rice technology provide an economically viable option for raising the grain yield and heterosis for economically important traits that enhance abiotic and biotic stress resistance and sustaining/securing growth in production and productivity. Hybrid rice technology pioneered in China is being adopted across the world with great success. Hybrid rice has the potential to increase yields by 15 to 20% over those of conventionally bred varieties (Virmani, 1994).

In hybrid breeding program, utilization of parental lines with considerable variability is of primary concern for exploitation of maximum level of heterosis or hybrid vigor in the F_1 seed production. In rice, cytoplasmic male sterile (CMS) based three-line breeding system has become the most popular method of hybrid rice production which has helped to evaluate more rice hybrids. DNA based molecular markers such as restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), randomly amplified polymorphic DNA (RAPD) (Ayesha et al., 2011;

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Table 1. List of genotypes used for analysis.

Accession ID	Characteristics	Source*
IR58025B	Maintainer	IRRI, Philippines
IR68888B	Maintainer	IRRI, Philippines
IR68897B	Maintainer	IRRI, Philippines
IR68902B	Maintainer	IRRI, Philippines
IR69624B	Maintainer	IRRI, Philippines
IR72078B	Maintainer	IRRI, Philippines
IR72080B	Maintainer	IRRI, Philippines
KMR-3	Restorer	UAS, Mandya, India
PRR-78	Restorer	IARI, New Delhi, India
BR-827-35	Restorer	KKV, Kajrat, India
NDR-3026	Restorer	NDUAT, Faizabad, India
IR40750	Restorer	IRRI, Philippines

*IARI: Indian Agricultural Research Institute; IRRI: International Rice Research Institute; KKV: Konkan Krishi Vidyapeeth; NDUAT: Narendra Dev University of Agricultural Sciences and Technology; UAS: University of Agricultural Sciences.

Williams et al., 1990), simple sequence repeats (SSR) (He et al., 2012; Kaladhar et al., 2004; Tautz, 1989) and amplified fragment length polymorphism (AFLP) (Vos et al., 1995) etc have been used effectively to assess the genetic variability in several crop species. It also facilitates identifying of parental line specific molecular markers (Nandakumar et al., 2004; Sarao et al., 2010), that are useful in detecting hybrids derived from them as well as genome mapping of economically important traits (Chen et al., 2011; Kumar et al., 2012). The present study was undertaken to evaluate the genetic variability among a set of elite hybrid rice parental lines with the objectives namely; (i) identification of parental line specific molecular tool and (ii) assessment of genetic variability among maintainer and restorer lines.

MATERIALS AND METHODS

DNA extraction

Twelve elite parental lines of commercial hybrid rice consisting of seven maintainers (B) and five restorers were used (Table 1). Seeds were procured from their sources as given in Table 1, grown in the field at Maharajpet farm of the Barwale Foundation, Hyderabad, India. Genomic DNA was extracted from four-week old rice seedlings using modified Dellaporta method (Dellaporta et al., 1983).

Polymerase chain reaction (PCR) with microsatellite markers

Genetic diversity analysis among selected rice lines was carried out using hundred rice microsatellite markers (McCouch et al., 2002) spanning 12 chromosomes of rice (Table 2). Markers were selected based on the relative length of the chromosomes. PCR reaction was carried out in 10 µl reaction volume containing 10 to 15 ng genomic DNA, 1× PCR buffer (10 mM Tris-Cl [pH 8.4]; 50 mM KCl), 200 µM dNTPs, 5 pM of each forward and reverse primer, 0.5U *Taq* polymerase enzyme. The PCR amplification was carried out in MJ Research thermal cycler. The profile comprised of initial denaturation step at 95°C for 5 min, cyclic denaturation step at 94°C for 8 s, primer annealing step at 55°C for 5 s and the primer extension step at 72°C for 40 s. The cycle was repeated 30 times and ended with the final extension at 72°C for 7 min. The PCR amplified products were resolved on 5% denaturing polyacrylamide sequencing gel stained with silver staining procedure, air dried and scanned for genotyping analysis.

Data analysis

Molecular data set was prepared by scoring the SSR markers amplification profile as present (1) or absent (0) of a specific band size and used as genetic characters to determine the relationship among the select lines. Using Numerical Taxonomy and Multivariate Analysis System (NTSTS) pc version 2.1, similarity indexes were calculated by the Similarity for Quantitative Data (SIMQUAL) subroutine. Clustering analysis and their corresponding dandrogram were generated using unweighted pair group mathematical average (UPGMA) cluster analysis (Rohlf, 2000).

RESULTS AND DISCUSSION

SSR marker polymorphism and cultivar specific fingerprint markers

Hundred SSR markers evenly spread across the rice genome was used for fingerprinting twelve elite hybrid rice parental lines. Amongst the markers screened, 62 were polymorphic, 32 were monomorphic and remaining 6 manifested null allelic. Polymorphic SSR markers generated 203 alleles with an average of 3.2 alleles per primer. RM562, RM589, RM541, RM440 and RM107 were found to be highly polymorphic with 5 to 6 alleles. The product sizes of the scored bands ranged from 150

Table 2. List of rice microsatellite markers screened.

S/N	Chr.1	Chr.2	Chr.3	Chr.4	Chr.5	Chr.6	Chr.7	Chr.8	Chr.9	Chr.10	Chr.11	Chr.12
1	RM 580	RM 521	RM 293	RM 417	RM 146	RM 587	RM 533	RM 531	RM 464	RM 591	RM 479	RM 155
2	RM 595	RM 475	RM 338	RM 124	RM 437	RM 588	RM 182	RM 325	RM 460	RM 496	RM 139	RM 491
3	RM 495	RM 424	RM 411	RM 401	RM 194	RM 589	RM 192	RM 195	RM 328	RM 484	RM 441	RM 101
4	RM 562	RM 341	RM 442	RM 185	RM 548	RM 541	RM 445		RM 107	RM 330	RM 181	
5	RM 297	RM 423	RM 468	RM 273	RM 592	RM 584						
6	RM 113	RM 266	RM 489	RM 280	RM 161	RM 549						
7	RM 129	RM 106	RM 503	RM 317	RM 291	RM 133						
8	RM 165		RM 514	RM 451	RM 305	RM 190						
9	RM 283		RM 517	RM 142	RM 430	RM 508						
10	RM 5		RM 546	RM 119	RM 440	RM 439						
11	RM 581			RM 127	RM 516	RM 539						
12	RM 428			RM 131								
13	RM 431			RM 177								
14	RM 513			RM 456								
15	RM 472			RM 518								
16	RM 529			RM 551								
17	RM 157			RM 559								
18	RM 265			RM 564								
19	RM 449											
20	RM 102											
21	RM 568											
# primers	**21**	**7**	**10**	**18**	**11**	**11**	**4**	**3**	**4**	**4**	**4**	**3**

Table 3. Identification of genotype specific SSR markers.

Accession ID	# unique markers detected	Genotype specific SSR markers*
IR58025B	4	RM529(1), 589(6), RM533(7), RM182(7)
IR68888B	8	RM580(1), RM595(1), RM562(1), RM113(1), RM129(1), RM449(1), RM541(6), RM441(11)
IR68897B	2	RM468(3), RM440(5)
IR68902B	1	RM549 (6)
IR69624B	2	RM521(2), RM305(5)
IR72078B	6	RM5(1), RM475(2), RM341(2), RM589(6), RM508(6), RM484(10)
IR72080B	5	RM341(2), RM514(3), RM442(3), RM456(4), RM589(6)
KMR-3	6	RM297(1), RM442(), RM456(4), RM541(6), RM584(6), RM107(9)
PRR-78	3	RM595(1), RM328(9), RM107(9)
BR-827-35	3	RM518(4), RM584(6), RM107(9)
NDR-3026	3	RM283(1), RM521(2), RM442(3)
IR40750	1	RM265(1)

*Numbers in parenthesis indicate specific rice chromosome.

to 300 bp. Most of the polymorphic primers sets identified in this study produced cultivar specific amplification profile (Table 3). RM 206, RM338, RM589, RM584 and RM549 produced monomorphic amplification profile among all maintainer lines but polymorphic with variable allele size among restorer lines.

Genetic relatedness based on SSR markers

UPGMA clustering diagram along with the genetic similarity index value generated for the genotypic dataset showed two major clusters (C1 and C2) corresponding to the maintainer (B) and restorer (R) groups with sub

clusters in each group. The overall genetic similarity index value ranged from 0.69 to 0.87 with the mean value of 0.78 indicated the mean genetic diversity of about 22% among the genotypes studied (Figure 1a). Cluster C1 consist of two sub clusters SM1 and SR1, of which SM1 contained five B-lines namely IR58025B, IR68897B, IR68902B, IR69624B and IR72078B that shared the genetic similarity of more than 80%; and the sub cluster SR2 included two R-lines BR-827-35 and IR40750 with the genetic similarity of about 75% (Figure 1a and b). Similarly, C2-cluster consisted of two sub clusters SR1 and SM2 with the genetic similarity of above 75 and 70% respectively. Sub cluster SR1 contained three R-lines namely KMR-3, PRR-78 and NDR-3026; of which two R-lines KMR-3 and PRR-78 were found to possess maximum level of 84% genetic similarity; and sub cluster SM2 contained two B-lines IR72080B and IR68888B (Figure 1a and c). Under the platform of union for the protection of new plant varieties (UPOV), plant breeders have the right and a privilege to protect their plant materials and also make it available for further crop improvement by others. India is the first in the world to implement similar rights to the farmers under the act “Plant Variety protection and Farmers act- 2001” (Sahai, 2003). In order to protect the plant materials, conventionally, DUS (Distinction, Uniformity and Stability) test was conducted in the field, which is a time consuming, labor intensive and laborious process. Development of DNA fingerprinting techniques enabled to generate such DUS data in a faster and efficient way as compared to morphological or biochemical markers. Besides helping varietal identification, DNA markers also have numerous other applications in crop genetics such as genetic diversity and relationships, tagging useful traits, assessing the genetic purity of the inbred lines, evolutionary studies, selection of recurrent parental genome in back cross breeding etc.

Successful hybrid breeding program lies on identifying the heterotic groups or parental line. DNA fingerprinting and genetic diversity of among hybrid rice parental lines were well documented in the past using simple sequence repeated (SSR) markers due to their characteristics such as being highly polymorphic, multi allelic and co-dominant (Duan et al., 2002; Jaikishan et al., 2006; He et al., 2012; Wang and Lu, 2006; Xu et al., 2002). In this study, hundred SSR markers were used to identify cultivar specific molecular markers as well as to assess the genetic diversity among maintainer and restorer lines of WA cytoplasm. Of 94 SSR markers amplified out of 100 markers studied, 62 produced more than one allele. It indicated about 66% of the used markers were polymorphic with two and more alleles. Identification of 203 polymorphic markers out of 235 amplified bands indicated the hyper variability of the SSR marker type as reported earlier (Akagi et al., 1997). Most of the markers generated cultivar specific fingerprint profile which is useful in authentication of cultivar(s), genetic purity of foundation seed stock and their hybrids with an example: commercial rice hybrid KRH-2 can be detected using its male parent KMR-3 specific markers RM297, RM442, RM541, RM584 and RM107 and female parent IR58025A specific markers RM529, RM489, RM589, RM533 and RM182 identified in this study. Hyper variable markers RM 206, RM338, RM589, RM584 and RM549 among restorer line in contrast to maintainer lines need further investigation with respect to the trait association.

Genetic relationship and clustering

The overall genetic distance of about 22% among commercial hybrid rice parental genotypes revealed that the genetic variability among these genotypes was considerably low. This result is in accordance with the previous reports of Kaladhar et al. (2004) and Xu et al. (2002). Clustering study clearly demarcated B and R lines into two separate groups (Figure 1a). It was consistent with the breeder’s practice of not making inter group crossing (Wang and Lu, 2006). Few exceptions were also noticed by clustering of lines with different genetic group (restorer line grouped with maintainer line and *vice versa*) which explained considerable degree of genetic relatedness among them as noticed earlier by Jaikishan et al. (2006) and Xu et al. (2002) and the possible reason could be due to the origin of their progenitors (Kaladhar et al., 2004). The mean genetic distance value of about 22% within B lines (Figure 1b) and 23% within R lines (Figure 1c) indicated these lines are genetically less diverse. It may be due to the use of limited germplasm sources for B line development. During the earlier stage of tropical hybrid rice breeding program at IRRI, several B lines were developed from single, elite and most popular B-line IR58025B, resulted in the load of genetic homogeneity (He et al., 2012). Since molecular markers are reliable in estimating the genetic diversity accurately by detecting variations at DNA sequence level and the pedigree records provide parentage information, combined use of them will greatly facilitate enhancement of the genetic diversity among B and R lines thereby leading to the development of heterotic pools and maximization of the heterosis in hybrid rice breeding program.

Conclusion

SSR markers screened among hybrid rice parental lines generated several cultivar specific molecular markers which are useful in varietal authentication and genetic purity assays. Genetic distance studies demonstrated low level of genetic variability existing among maintainer and restorer lines of commercially useful WA-CMS, suggesting the need to utilize diverse genetic resources as hybrid rice parental lines in order to maximize heterosis

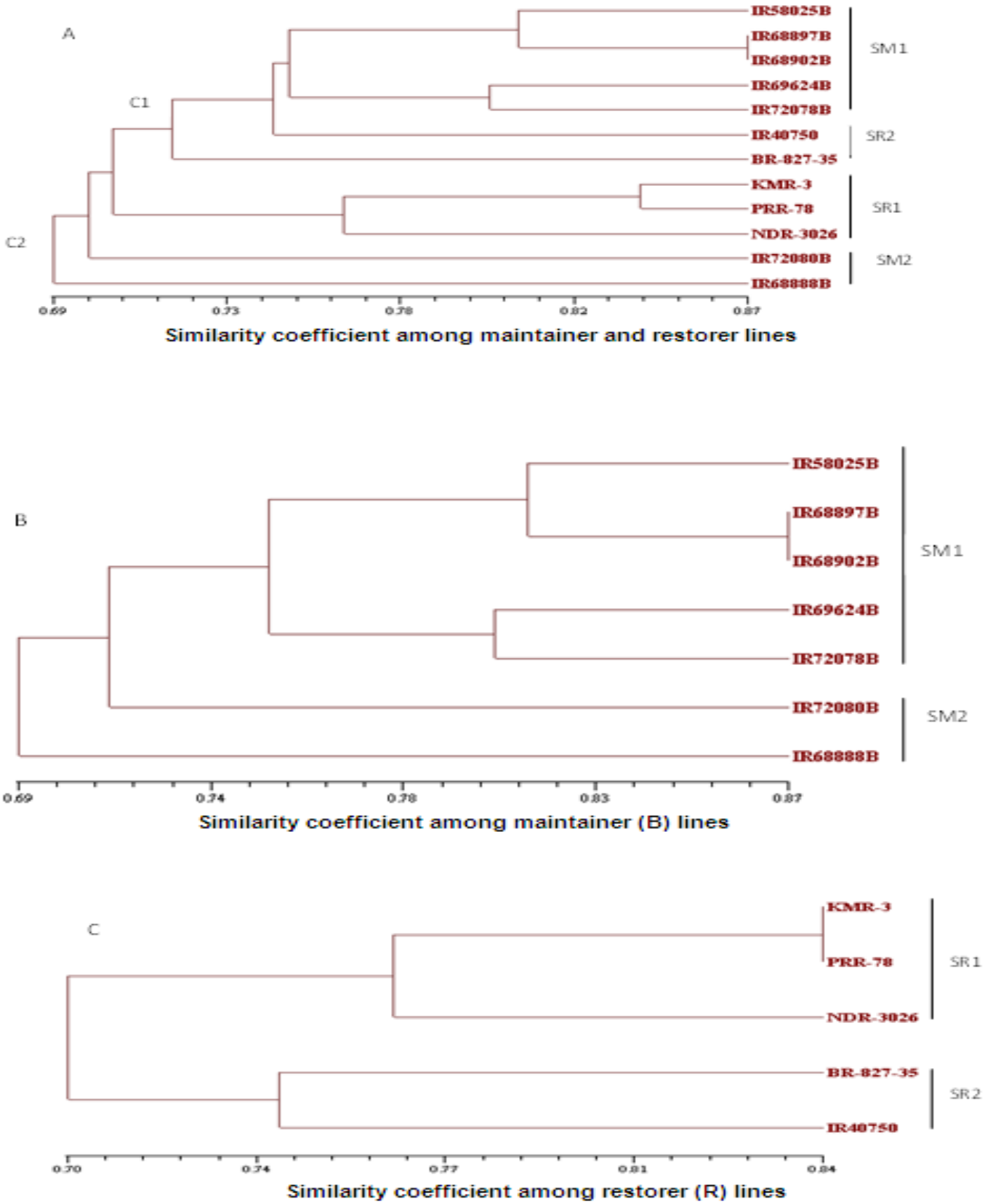

Figure 1. Cluster diagram based on genetic similarity analysis among maintainer and restorer rice lines using NTSYS software program.

in hybrid rice breeding.

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