Selection of Elite Maintainers with *eui* Gene using RAPD Analysis for Development of New CMS Lines in Hybrid Rice Breeding

Mayank Rai[1], MP Pandey*, Pankaj Sharma[1], Devanshi[2], Lalit Arya[3] and Rakesh Singh[3]
[1]*Department of Genetics and Plant Breeding, GB Pant University of Agriculture & Technology, Pantnagar-263145 (Uttaranchal), India*
[2]*Department of Genetics, Indian Agricultural Research Institute, New Delhi-110012, India*
[3]*NRC on DNA Fingerprinting, National Bureau on Plant Genetic Resources, New Delhi-110012, India*

Random Amplified Polymorphic DNA (RAPD) analysis was employed for genetic diversity study over 32 rice genotypes including 29 agronomically superior maintainer lines with *eui* gene for good panicle exertion and 3 restorers. A total of 102 amplification products were detected with 12 polymorphic RAPD primers of which 69 (67.64%) were polymorphic. Polymorphic primer showed 50 to 80 amplicon polymorphism. The genetic similarity ranging between 54.2% (L_{19}-L_5) to 92% (L_{11}-L_2) suggested that the maintainers and restorers under study are divergent. Cluster generated through UPGMA analysis revealed two main clusters. Cluster I comprised two genotypes only, both derived from the same cross. Genotypes in cluster II were redistributed into a number of subclusters at different similarity coefficient levels indicating different levels of genetic relationships. The maintainer lines L_2, L_{14}, L_{17}, L_{20} and L_{29} used in the study are suggested to be exploited for development of CMS lines to realize higher level of heterosis. The hybrids involving parents with maximum dissimilarity coefficients displayed non-significant estimates of heterosis for grain yield per plant.

Key Words: Rice, RAPD, WA-CMS, Heterosis

Introduction

Rice is grown in 114 countries across the world, on 150 million hectare area that constitutes nearly 11% of the world cultivated land (Rai, 2006). Exploitation of heterosis is the most pragmatic approach to increase the rice yield potential. However, the success is determined not only by the performance of the parents *per se* but also by the divergence between them (Yuan *et al.*, 1994; Gopal and Minocha, 1997). Molecular markers based on polymerase chain reaction (PCR) method offer several advantages over the conventional morphological markers. Random Amplified Polymorphic DNA (RAPD) uses arbitrary 10-base primers to amplify the random portions of the genome (Williams *et al.*, 1990; Naghia *et al.*, 2002). The fragments produced are easily visualized on ethidium bromide stained gel and polymorphism can be detected between the amplification products of different individuals. The level of correlation between molecular marker based distances and hybrid performance is dependent on the germplasm (Saghai-Maroof *et al.*, 1997). The RAPD analysis (Williams *et al.*, 1990) being relatively simple and based on the use of commercially available random primers provides useful information about the molecular distance within and among varieties.

Since most of the three-line hybrids either already released or in the advanced trials since start of the programme in 1994 utilize IR58025A as the sole CMS line with wild abortive (WA) cytoplasm, therefore, the extent of exploitable heterosis is limited to a point. Correlation of molecular diversity with heterosis reflects usefulness of RAPD in predicting heterosis based on RAPD marker polymorphism (Xiao *et al.*, 1996). We report here the genetic divergence among the maintainer lines specifically bred for improved plant type, duration, yield potential and grain quality at the GB Pant University of Agriculture and Technology, Pantnagar and three adapted varieties/ restorers by employing RAPD markers. This will aid in finding new vistas to develop CMS lines in diverse genetic backgrounds for the greater exploitation of heterosis in hybrid rice improvement programme.

Materials and Methods

Plant Material

Plant material used for this study consisted of 32 rice genotypes (Table 1) including 29 improved maintainer lines with good yielding ability and other agronomic characters that were bred specifically for the development of superior cytoplasmic male sterile lines. The elite lines were also selected for better panicle exertion as one of the parents in the cross involved '*eui*', the single recessive gene for elongated uppermost internode. The material also included three widely adapted superior genotypes being used as restorers in our hybrid breeding programme *viz.*,

---

**Corresponding author: matappandey@rediffmail.com*

J. Plant Genet. Resour. 20(2): 98-103 (2007)

Table 1. Parentage of the elite maintainer lines and restorer genotypes

S.No.	Designation	Code	Parentage
1.	UPR 2899-78-4-2B	L_1	UPRI 95-140/IR Basmati
2.	UPR 2899-78-4-3B	L_2	-do-
3.	UPR 2899-78-5-1B	L_3	-do-
4.	UPR 2899-78-6-2B	L_4	-do-
5.	UPR 2900-80-1-1B	L_5	Haryana Basmati 1/UPRI 95-139
6.	UPR 2906-254-2-6-5B	L_6	Govind/UPRI 95-139
7.	UPR 2906-257-2-8-1B	L_7	Haryana Basmati 1/UPRI 95-139
8.	UPR 2911-271-2-4B	L_8	UPRI 95-139/IR 58025B
9.	UPR 2911-271-2-6B	L_9	-do-
10.	UPR 2911-271-3-2B	L_{10}	-do-
11.	UPR 2911-271-7-1B	L_{11}	-do-
12.	UPR 2911-271-8-15B	L_{12}	-do-
13.	UPR 2911-271-8-3B	L_{13}	-do-
14.	UPR 2911-271-8-4B	L_{14}	-do-
15.	UPR 2911-271-8-9B	L_{15}	-do-
16.	UPR 2911-271-12-2B	L_{16}	-do-
17	UPR 2912-273-8-1B	L_{17}	UPRI 95-141/IRBB 21
18.	UPR 2900-80-1-4B	L_{18}	Haryana Basmati 1/UPRI 95-139
19.	UPR 2900-80-9-2-1B	L_{19}	-do-
20.	UPR 2900-80-9-2-2B	L_{20}	-do-
21.	UPR 2903-87-1-1B	L_{21}	Pusa Basmati 1/IR 58025B
22.	UPR 2903-87-1-2B	L_{22}	-do-
23.	UPR 2904-207-1-3B	L_{23}	BG 1321/UPRI 95-141
24.	UPR 2906-254-2-6-2B	L_{24}	Govind/UPRI 95-139
25.	UPR 2911-271-2-1B	L_{25}	UPRI 95-139/IR 58025B
26.	UPR 2911-271-2-5B	L_{26}	-do-
27.	UPR 2911-271-3-3B	L_{27}	-do-
28.	UPR 2911-271-8-7B	L_{28}	-do-
29.	UPR 2911-271-12-3B	L_{29}	-do-
30.	UPRI 93-287	T_1	MRC 19340
31.	Pant Dhan 4	T_2	IR 262/Remajda
32.	Ajaya	T_3	IET 4141/CR 98-7216

Pant Dhan 4, Ajaya and UPRI 93-287, which have shown fertility restoration capability to wild abortive (WA) based cytoplasmic male sterility.

During 1998-1999 off season, all the 29 maintainers were crossed to UPR 93-287 and Pant Dhan 4 in line x tester fashion. To understand the effect of diversity on the extent of heterosis in crosses, the complete set of hybrids and parents were evaluated in the randomized complete block design (RCBD) using two replications and under two different environments based on fertility. The environments were created using two fertilizer doses *viz.*, optimum (120N: 40P: 40K kg/ha) and high (180N: 80P: 40K kg/ha) fertility, referred as E_1 and E_2, respectively. Data on yield related characters were obtained to calculate heterosis.

DNA Extraction

For each genotype, 100 mg of fresh leaves from four-week old seedlings were weighed for DNA extraction using CTAB miniprep DNA extraction protocol (Saghai-Maroof *et al.*, 1984). DNA was quantified in a TKO 100 Fluorometer (Hoefer, San Fransisco, CA).

RAPD Analysis

A total of 70 RAPD primers (A, B, C, D and E series) were initially taken (Operon Technology Alameda, California, USA) for this study. Amplifications were carried out with 1x PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2% formamide), 1.5 mM $MgCl_2$, 200 μM dNTPs, 200 nM primer, 1 unit of *Taq* DNA polymerase (Bangalore Genei, India) and 25 ng of genomic DNA. Amplifications were performed using a 96-well thermal cycler (Perkin-Elmer, USA) programmed for an initial step of 5 min at 94°C followed by 45 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C. A final extension step at 72°C was programmed for 7 minutes. Amplification products were separated on 1.4% agarose gels prepared in 1x TAE buffer (40 mM Tris-acetate and 1 mM EDTA pH 8.0) at 60 V for 3 h using a horizontal gel electrophoresis system (Bio-rad, Hercules, CA, USA). Gels were stained with ethidium bromide. A 100 bp DNA ladder plus (MBI, Fermentas, Burlington, Ontario, Canada) was run alongside the amplified products to determine their approximate size. Gel was photographed using Gel-Documentation system (Gel Doc Mega, Biosystematica, UK).

Data Analysis

Reproducible amplified fragments of RAPD were scored manually. Weak bands of negligible intensity and smeared bands were excluded from the final data analysis. Band profiles were scored with 1 indicating the presence, and 0 indicating the absence of a band to construct a binary qualitative data matrix. Pair-wise comparisons of genotypes were employed to calculate Jaccard's similarity coefficient (GS): a / (n-d), where a = number of positive matches; d = number of negative matches and n = total sample size (Jaccard, 1908).

A dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) and computation for multivariate analysis was done using the computer programme NTSYS-pc Version 1.8 (Rholf, 1993).

Statistical analysis was carried out to assess the heterosis as per Fonseca and Patterson (1968). Pant Dhan 4, a widely cultivated variety was taken as check to calculate standard heterosis.

Results and Discussion

RAPD Analysis

In the present study, band profiling for the genotypes was done with replicated PCR amplification to check the reproducibility of the technique. Out of 70 RAPD primers, only 12 primers with clear and reproducible band pattern were chosen for further study. Details of the polymorphic RAPD primers and the level of polymorphism are presented in Table 2.

Twelve polymorphic primers (Table 2) generated 102 fragments (8.5 bands per primer) among all the 32 genotypes, of which 67.64% (69 bands) were polymorphic for one or more genotypes. Similar level of polymorphism (69.4%) using RAPD markers have been reported by He *et al.* (2004) based on the genetic diversity study on allelopathic rice germplasm. The number of polymorphic fragments ranged from 4 to 10 (5.75 average). None of the primers revealed 100% polymorphism; however, the primers OPA-19, OPB-17 and OPE-09 detected 75% or higher polymorphism. The distinct banding patterns as produced by the primer OPE-09 (Figs. 1a and b) are adequate enough to differentiate all the 32 genotypes at molecular level. These results reflect that RAPD is technically simpler, quicker, relatively inexpensive and non-radioactive as also observed by Samec (1993). The results suggest that RAPD is suitable marker system for DNA fingerprinting and diversity analysis at whole genome level with minimum inputs, in contrast to the most popular STMS markers which is single locus, therefore needed in more number to generate information about rice fingerprint. RAPDs have been widely used to detect polymorphism in germplasm characterization and genetic distance studies (Naghia *et al.*, 2002; He *et al.*, 2004) and population differentiation (Yu *et al.*, 2005).

Genetic Diversity Based on RAPD Markers

Analysis of the relationship based on 102 amplified products (RAPD markers) through UPGMA analysis revealed that the genetic relationship based on Jaccard's coefficients ranged between 54.2% (L_{19}-L_5) to 92% (L_{11}-L_2). The dendrogram (Fig. 2) revealed 2 major clusters. Cluster I comprised of lines L_{19} and L_{20} (81.1% genetically similar), whereas, Cluster-II contained all the remaining 30 genotypes, including three improved cultivars *viz.*, T_1, T_2 and T_3. Both of the lines (L_{19} and L_{20}) in cluster-I were derived from a single cross Haryana Basmati1 × UPRI 95-139. All other lines derived from this cross grouped in different sub clusters of cluster II at different genetic similarity levels. The cluster-II was sub grouped into 5 sub clusters (IIA-IIE). Sub-clusters IIA and IIB were comprised of two genotypes each whereas, sub-cluster IIC comprised of four genotypes. In sub-cluster IID highest number of genotypes i.e. 14 genotypes were grouping together whereas, in sub-cluster IIE six genotypes were grouping together. The genotypes L7 and L18 were ungrouped in the sub-clusters. Amongst the restorers, T_1 (UPRI93-287) was closer to most of the elite maintainer lines in comparison to Pant Dhan 4 (T_2) and Ajaya (T_3). The lines T_2 and T_3 were 79.4% similar. It suggests the presence of high level of genetic divergence in the plant material under study and therefore, enhanced prospects of obtaining higher standard heterosis by crossing restorers to elite maintainer converted into cytoplasmic male sterile lines. Two different lines L_2 (UPRI 95-140 / IR Basmati) and L_{11} (UPRI 95-139 / IR

Table 2. RAPD Primers along with their sequences and level of polymorphism detected

Primer Designation	Sequence (5′ - 3′)	Total number of bands	Polymorphic loci	
			Frequency	(%)
OPA-04	AATCGGGCTG	8	5	62.50
OPA-08	GTGACGTAGG	5	4	80.00
OPA-19	CAAACGTCGG	9	7	77.77
OPB-10	CTGCTGGGAG	8	5	62.50
OPB-17	AGGGAACGAG	4	3	75.00
OPD-03	GTCGCCGTCA	10	7	70.00
OPD-08	GTGTGCCCCA	8	4	50.00
OPE-03	CCAGATGCAC	6	4	66.66
OPE-07	AGATGCAGCC	6	3	50.00
OPE-04	TGCGGCTGAG	11	8	72.72
OPE-09	ACGGCGTATG	13	10	76.92
OPE-03	GGCTGCAGAA	14	9	64.28
	Total	102	69	–
	Average		5.75	67.64

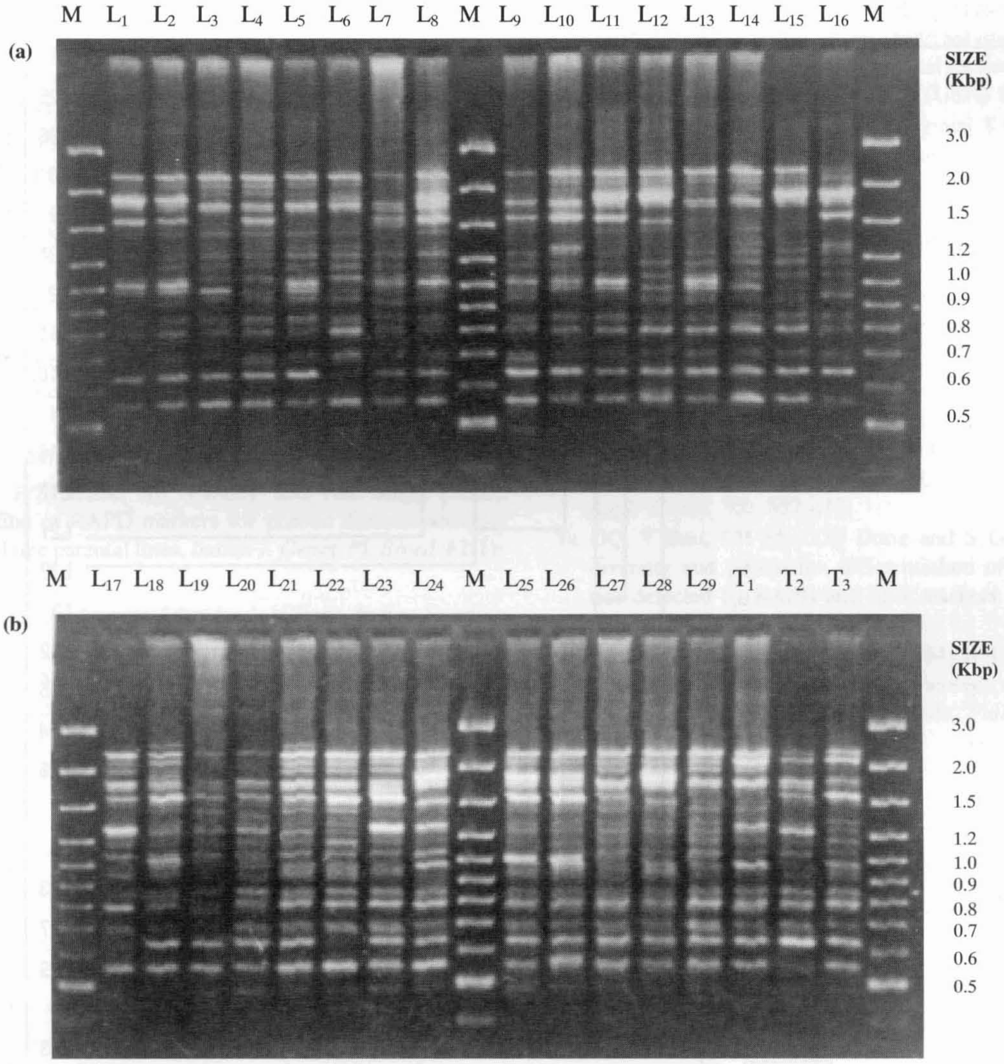

Fig. 1: RAPD profile of 29 maintainer lines (L_1-L_{29}) and three improved cultivars of rice with primer OPE-09, Lane M: DNA size marker "100 bp DNA ladder plus"

58025 B), although of different origin were close to each other with the genetic distance of 0.08 (92% similarity). Interestingly, the study also revealed the intermingling of the lines originating from different crosses into the same cluster and vice-versa. This could be due to the fact that some of the lines had one of the parents common in these crosses. For example, 12 of the lines originating from two crosses *viz.*, L_8 to L_{16} and L_{25} to L_{29} (UPRI 95-139 × IR 58025B) and L_{21}-L_{22} (Pusa Basmati 1 × IR58025 B) involved IR58025B as common parent. Similarly, the lines L_5, L_7 and L_{18} to L_{20}, derived from the cross Haryana Basmati 1 × UPRI 95-139 and the lines L_8 to L_{16} and L_{25} to L_{29} derived from the cross UPRI 95-139 × IR 58025B, UPRI 95-139 is common. Within the cluster- II, the lines derived from UPRI 95-139 × IR 58025B (L_8 to L_{16}) and L_{25} to L_{29} were grouped into five different sub-clusters, based on their relative genetic similarity. Such a variation could be attributed to the selection of diverse plant characteristics related to plant type, duration, grain and yield attributes during segregating generations (F_2-F_4) leading to divergence among these lines.

The present study showed higher level of heterosis for yield and related attributes with L_2, L_{14}, L_{17}, L_{20} and L_{29} crossed with T_1/T_2 (Table 1). The result suggested use of these maintainer lines for CMS line development to realize higher heterosis in hybrid rice development. Linear relationship between heterosis of specific crosses and divergence of combining parents in this study could not be established as also reported by Dudley *et al.* (1991), since the primers used were random and limited, so may

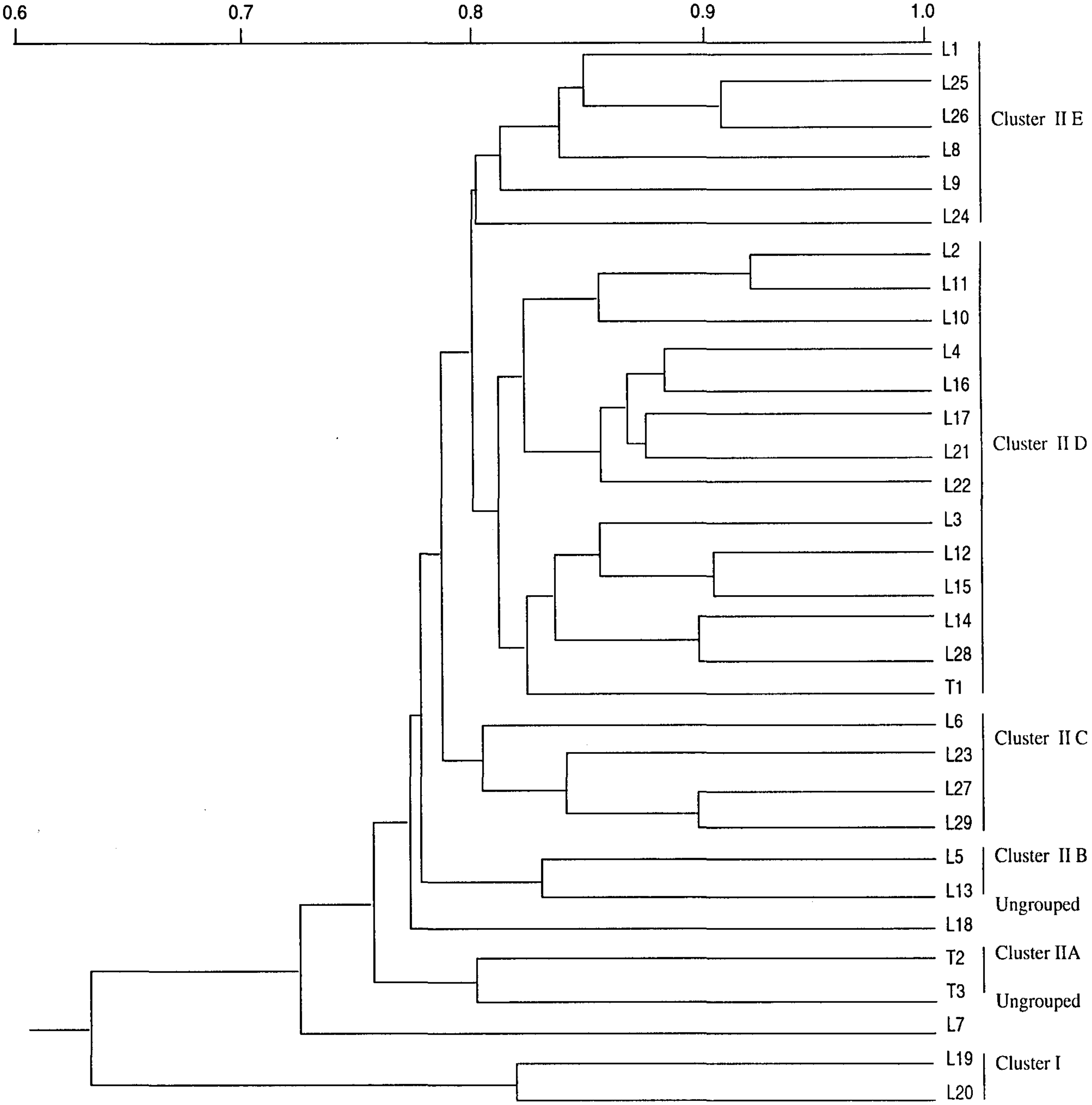

Fig. 2: Dendrogram generated based on RAPD primers for 29 maintainer lines and three improved cultivars of rice

not be able to cover the complete genome. The results however indicated top heterotic crosses for grain yield per plant and other characters revealing low level of genetic diversity between parents. On the other hand, the hybrids involving parents with maximum dissimilarity coefficients (L_{19} x T_2) with genetic similarity of 62.1%, displayed non-significant estimates of heterosis (-19.89) for grain yield per plant. It suggested the prospects of higher level of heterosis with parents having optimum level of diversity.

In the present study, the RAPD markers had revealed the genetic diversity among lines and grouped them into distinct clusters and subclusters. The findings will have immediate practical implication in the hybrid breeding programme for the development of CMS lines of varying duration, plant height and productivity traits with optimum genetic diversity, which can exhibit enhanced level of heterosis. The present study emphasizes the use molecular markers in parental improvement and development of heterotic rice hybrids.

References

Dudley JW, MAS Maroof and GK Rufener (1991) Molecular markers and grouping of parents in maize breeding programme. *Crop Sci.* **31:** 718-723.

Fonseca S and FL Patterson (1968) Hybrid vigour in a seven parent diallel cross in common winter wheat (*Triticum aestivum* L.). *Crop Sci.* **8:** 85-88.

Gopal J and JL Minocha (1997) Genetic divergence for cross prediction in potato. *Euphytica* **97:** 269-275

He HQ, XL Jia, YY Liang, LH Shen, BQ Song, YC Guo, KJ Liang and WX Lin (2004) Assessment of genetic diversity of allelopathic rice germplasm based on RAPD and ISSR. *Yi Chuan Xue Bao* **31(9):** 888-894.

Jaccard P (1908) Nouvelles recherches sur la distribution forale. *Bull. Soc. Sci. Nat.* **44:** 223-270.

Naghia PT, JPS Malik, MP Pandey and NK Singh (2002) Application of RAPD markers for genetic distance analysis of hybrid rice parental lines. *Indian J. Genet. Pl. Breed.* **62(1):** 1-4.

Rai M (2006) Rice–the cereal that feeds billions. *Indian Farming* **56(7):** 4-9.

Rohlf FJ (1993) NTSYS-pc Numerical taxonomy and multivariate analysis system, version 1.80 Exeter Software, Setauket, New York.

Saghai-Maroof AM, KM Soliman, RA Jorgesen and RW Allard (1984) Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci.* (USA) **81:** 8014-8018.

Saghai-Maroof AM, GP Yang, Q Zang and KA Gravois (1997) Correlation between molecular marker distance and hybrid performance in US Southern long grain rice. *Crop Sci.* **37:** 145-150.

Samec P (1993) DNA polymorphism and RAPD technology. *Gene Slecht* (Prague) **29:** 291-320.

Williams JGK, AR Kubelik, KJ Livak, JA Rafalski and SV Tingey (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* **18:** 6531-6535.

Xiao J, J Li, I Yuan, SR McCouch and SD Tunksley (1996) Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR based markers. *Theor. Appl. Genet.* **92:** 637-643.

Yu GQ, Y Bao, CH Shi, CQ Dong and S Ge (2005) Genetic diversity and population differentiation of Liaoning weedy rice detected by RAPD and SSR markers. *Biochem. Genet.* **43:** 261-70.

Yuan LP, ZY Yang and JB Young (1994) Hybrid rice in China: In: SS Virmani (ed.) *Hybrid Rice Technology, New developments and Future Prospects.* IRRI, Manila, Philippines.