RESEARCH ARTICLE

Determination of genetic relationships among elite thermosensitive genic male sterile lines (TGMS) of rice (*Oryza sativa* L.) employing morphological and simple sequence repeat (SSR) markers

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Abstract

A set of morphological traits and SSR markers were used to determine the genetic relationship among 12 elite thermosensitive genic male sterile (TGMS) lines developed at three different research institutions of India. Agro-morphological data recorded on 20 morphological traits revealed a wide base of genetic variation and a set of four morphological traits could distinguish most of the TGMS lines. Analysis with 30 SSR markers (20 EST-SSRs and 10 genomic SSRs) revealed 27 markers to be polymorphic, amplifying a total of 83 alleles. Each SSR marker amplified 2–6 alleles with an average of 2.76 alleles per marker and a PIC value varying from 0.54 to 0.96. Cluster analysis based on SSR and morphological data clearly differentiated the lines according to their source of origin. Correlation analysis between morphological and molecular data revealed a very poor association ($r = 0.06$), which could be attributed to selection pressure, genetic drift, sampling error and unknown relationship among related lines. The SSR markers discriminated the genotypes distinctly and quantified the genetic diversity precisely among the TGMS lines. Data on the yield per plant indicated that the genotypes grouping under a similar cluster showed same heterotic behaviour as compared to the genotypes from different clusters when crossed to similar pollinators.

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Introduction

Rice is grown worldwide on about 154 million hectares annually with a total production of 600 million tons and accounts for 35%–75% of the total calories consumed by more than 3 billion Asians (Khush 2005). To feed an estimated 5 billion rice consumers by 2025, rice varieties with higher yield potential and greater yield stability need to be developed (Khush 2005). Rice is a staple food crop of India and hybrid rice technology provides a practically feasible approach to break the yield ceiling, thereby meeting the demand of ever increasing population and sustaining the present status of self-sufficiency.

Though cytoplasmic male sterile (CMS) system or three-line system of hybrid rice breeding is by far the most widely used system in China and elsewhere, it has a major drawback of restricting the use of rice germplasm to only 20–30% for want of effective maintainer or restorer lines among rice varieties. Besides, the present three-line hybrids have moderate yield advantage of 15–20% over the HYVs. To overcome these constraints, a novel genetic male sterility system called thermosensitive genic male sterility (TGMS) can be deployed for development of two-line hybrids. TGMS was discovered in China (Sun *et al.* 1989), Japan (Maruyama *et al.* 1991) and at International Rice Research Institute (IRRI, Philippines) (Virmani and Voc 1991). In this system, sterility and fertility expression of a genotype is determined by temperature. Based on TGMS system, hybrids developed are called as two-line hybrids. Two-line hybrids are reported to possess higher magnitude of heterosis. TGMS system can be useful in tropical countries like India where substantial temperature variations are available between Rabi and Kharif

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seasons and between plains and hills. TGMS gene can be introgressed into any rice variety, while at the same time any rice variety can be used as male parent, thus giving a much wider choice for developing hybrids of desired types. Keeping these in view, TGMS system is considered to be an effective alternative to CMS system. The International Rice Research Institute (IRRI), Philippines; Directorate of Rice Research (DRR), Hyderabad, India; and Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, India, have developed many TGMS lines. Most of these lines have been developed using TGMS donor lines from IRRI in different genetic backgrounds. No information as yet is available regarding the levels of variability among different TGMS lines at the morphological or molecular level. Therefore, the present study was undertaken to characterize 12 TGMS lines developed at DRR, GBPUAT and IRRI using morphological traits, quantify the level of genetic diversity and to predict the heterotic behaviour based on their clustering pattern.

Materials and methods

Plant materials used for molecular and morphological characterization

Among the 12 TGMS lines used in the study, seven were from DRR, three from IRRI and two from GBPUAT.

Table 1. Evaluation of 20 morphological traits of the TGMS lines under study.

Traits/TGMS lines	DRR-1S	DRR-2S	DRR-3S	DRR-4S	DRR-5S	DRR-7S
Plant height (cm)	47.00	77.00	77.00	86.00	55.00	76.00
Number of tillers	24.00	23.00	29.00	18.00	25.00	20.00
Number of effective tillers	16.00	14.80	16.00	14.40	16.80	10.40
Panicle length (cm)	17.72	19.9	22.72	24.5	24	23.18
Panicle type	Partially open	Compact	Compact	Compact	Partially open	Partially open
Panicle exertion %	71.25	81.22	82.31	79.01	70.23	79.67
Glume angle (degree)	25	30	26	25	25	22
Grain type	Medium bold	Medium bold	Medium bold	Medium bold	Medium bold	Long bold
Apiculus pigmentation	Purple	Absent	Purple	Absent	Absent	Absent
Awning	Awnless	Awnless	Awnless	Awnless	Awnless	Awned
Stigma colour	Purple	White	Purple	Light purple	White	Yellow
Number of spikelets	63	165	139	199	158	140
Stigma exertion %	42.20	11.40	48.00	15.07	25.04	29.01
Flag leaf length (cm)	15.50	10.60	25.16	22.74	25.00	22.42
Flag leaf width (cm)	0.9	0.9	1.0	1.2	1.3	0.9
Grain length (mm)	3.0	3.0	3.5	3.1	3.0	4.0
Grain width (mm)	0.7	0.6	0.6	0.6	0.6	0.6
Anthesis time (duration)	9:20–12:15	10:10–12:15	9:25–12:20	9:25–12:25	9:36–12:15	9:35–12:15
Anthesis duration (min.)	175	125	175	180	159	158
Days to 50% Flowering (days)	77	94	92	94	106	91
Traits/TGMS lines	DRR-9S	IR70978-22S	IR73827-23S	IR73834-21S	UPRI-95-140S	UPRI-95-167S
Plant height (cm)	79.00	72.00	51.00	48.00	57.00	58.00
Number of tillers	29.40	17.40	27.20	26.60	29.60	29.60
Number of effective tillers	14.20	11.00	25.40	20.40	27.00	27.00
Panicle length (cm)	22.4	22.05	21.28	25	16.75	18.48
Panicle type	Compact	Compact	Partially open	Partially open	Open	Partially open
Panicle exertion %	82.88	87.91	88.01	72.23	74.44	77.28
Glume angle (degree)	20	25	24	20	20	20
Grain type	Long bold	Medium bold	Medium bold	Long slender	Medium bold	Medium bold
Apiculus pigmentation	Absent	Absent	Absent	Absent	Purple	Purple
Awning	Awned	Awnless	Awnless	Awned	Awnless	Awned
Stigma colour	Light purple	Yellow	purple	White	Purple	Purple
Number of spikelets	160	183	93	109	114	85
Stigma exertion %	27.98	37.46	31.18	34.41	60.00	71.00
Flag leaf length (cm)	20.94	26.50	17.58	22.17	18.72	19.72
Flag leaf width (cm)	0.9	0.9	0.7	0.7	0.8	0.8
Grain length (mm)	4.0	3.1	3.1	4.5	3.1	3.1
Grain width (mm)	0.5	0.5	0.6	0.4	0.6	0.6
Anthesis time (duration)	9:20 – 1:25	9:25 – 1:00	9:30 – 1:10	9:32 – 12:55	9:40 – 1:15	9:42 – 1:25
Anthesis duration (min.)	245	215	220	203	205	223
Days to 50% Flowering (days)	95	110	100	105	95	96

Morphological traits, 20 viz., plant height, number of tillers and effective tillers, panicle and spikelets per plant, panicle and stigma exertion percentages, angle of glume opening, flag leaf length and width, awning, stigma and apiculus pigmentation, panicle type and length, days to 50 per cent flowering, anthesis time and duration, grain length and width (table 1) were recorded and evaluated as per the standard evaluation system developed at IRRI (1996).

DNA extraction, PCR amplification and electrophoresis

Plant DNA was extracted from fresh young leaves as following the protocol of Dellaporta *et al.* (1983). The quality and quantity of DNA was evaluated by using a UV-spectrophotometer (Beckman, Brea, USA). The DNA samples were diluted to 10 ng/μL. A set of 20 EST-derived SSR primer pairs along with 10 non-EST SSRs were used in the present study. These were selected based on their high polymorphism information content (PIC) values and uniform distribution across the rice genome (table 2) based on information available at http://www.gramene.org. DNA samples (40 ng) were amplified in 25-μL reaction volumes containing 1× PCR buffer (10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (v/v) gelatin) (Bangalore Genei, Bangalore, India), 0.2 mM of each dNTPs (Bangalore Genei, Bangalore, India), 10 pmol of each primer and 1 U of *Taq* polymerase (Bangalore Genei, Bangalore, India). PCR was carried out in a Thermal cycler (Perkin-Elmer-Gene Amp PCR System 9700, USA). The basic PCR profile was 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C and 7 min at 72°C for final extension. PCR-amplified products were resolved on 4% agarose gels, stained with ethidium bromide and visualized under UV in a gel documentation system (Alpha Innotech, San Leandro, USA). The sizes of the amplified fragments were estimated with the help of software utility of the gel documentation system using 50-bp and 100-bp DNA ladder (MBI Fermentas, Maryland, USA) as the size standard.

Statistical analysis

All the morphological traits were standardized by subtracting the mean value and dividing by the standard deviation before calculating Euclidian distances. Based on standardized traits value, Euclidian distance (md_{ij}) between lines were calculated, and matrix of these values denoted as MD. Morphological similarities (ms_{ij}) were also calculated as ($1 - md_{ij}$) and matrix of these values was denoted as MS. Using the matrix

Table 2. List of SSR markers used for molecular characterization of 12 TGMS line.

Chromosome number	Marker name	Type of marker	Product range size (bp)	Position on chromosome (cM)	Expected PIC value
1	RM128	EST	148–166	134.8	0.63
1	RM104	EST	222–238	186.6	0.62
1	RM499	Non EST	80–150	0.0	0.79
2	RM154	EST	165–199	4.8	0.80
2	RM424	Non EST	239–290	66.0	0.76
2	RM535	Non EST	138–410	195.7	0.66
3	RM168	EST	96–116	171.2	
4	RM119	EST	166–172	76.1	0.56
4	RM127	EST	209–206	150.1	0.57
4	RM177	EST	192–195	50.8	0.36
4	RM335	Non EST	104–155	21.5	0.84
5	RM164	EST	246	78.7	NA
5	RM161	EST	165–183	96.9	0.64
5	RM169	EST	164–194	57.9	0.71
5	RM153	EST	189–204	0.0	0.64
5	RM163	EST	124	78.7	NA
5	RM592	Non EST	210–400	7.4	0.79
6	RM111	EST	118–126	35.3	0.54
6	RM136	EST	98–104	51.2	0.62
6	RM190	EST	104–124	7.4	0.80
6	RM204	Non EST	106–194	25.1	0.83
6	RM276	Non EST	85–153	40.3	0.84
7	RM70	Non EST	134–215	64.6	0.86
7	RM336	Non EST	148–193	61.0	0.79
7	RM418	Non EST	253–304	42.1	0.82
8	RM149	EST	243–303	103.7	0.73
9	RM160	EST	86–130	82.4	0.87
11	RM167	EST	127–159	37.5	NA
11	RM144	EST	214–255	123.2	0.76
11	RM287	Non EST	98–118	68.6	0.83

NA, not available.

MS = (1 – MD), UPGMA cluster analysis was performed using the statistical package NTSYS-PC 2.0 (Rohlf 1993), producing a dendrogram and principal component analysis (PCA) map depicting the relationship among the lines relative to the morphological characteristics. The resulting dendrogram was supported by calculating the cophenetic correlation coefficient (using Microsoft Excel package), which is a measure of the agreement between the similarity values implied by the dendrogram and those of the original similarity matrix. As regards to SSR marker analysis, PIC value for each SSR marker was calculated using the formula:

$$\mathrm{PIC} = \sum \left\{1 - P^2\right\} / n,$$

where P, frequency of band; n, number of alleles. To ascertain the statistical strength of genetic relationships identified through this analysis, bootstrapping of data (1000 per mutations) was performed. The similarity matrices were used to construct a dendrogram for all genotypes using SAHN of NTSYS-PC based on UPGMA. The fit of UPGMA cluster to the original similarity indices was computed according to the mantel test procedure by MXCOMP of the software package NTSYS-PC version 2.0 (Rohlf 1993).

Generation and evaluation of hybrids and correlation analysis

Based on the clustering pattern, four TGMS lines, two from each cluster (DRR-1S, IR73827-23S, UPRI-95-140S and UPRI-95-167S) and three pollinators (Shalivahana, BCW-56 and JGL-384) were crossed to produce a total of 12 hybrids. The F_1s were evaluated in three replications along with the parental lines. Data on yield performance was recorded in five plants from each replication.

Results

Analysis of TGMS lines on the basis of morphological traits

Based on the four morphological traits, a key descriptor was developed for identifying and characterizing the 12 TGMS lines (table 3). On the basis of panicle type and stigma colour, the TGMS lines DRR-9S, DRR-2S and IR70978-22S were clearly differentiated from the rest of the lines. The lines DRR-1S and IR73827-23S were grouped into one cluster but based on the apiculus pigmentation, both genotypes were clearly differentiated. Lines DRR-5S and IR73834-21S were grouped together based on the panicle type and stigma colour but could be differentiated based on the awning pattern. Similarly, the TGMS lines UPRI-95-140S and UPRI-95-167S were grouped together initially but based on the awning pattern, they were clearly differentiated. The last group that consisted of three TGMS lines DRR-3S, DRR-4S and DRR-7S were differentiated on the basis of apiculus pigmentation and awning, indicating the importance of the morphological descriptors in differentiating genotypes.

Genetic similarity and cluster performance on the basis of morphological traits

The average genetic similarity (GS) among the TGMS lines on the basis of morphological traits after standardization was 0.54 and the value ranged from 0.89 (between UPRI-95-140S and UPRI-95-167S) to 0.19 (between four pairs of

Table 3. Genotype differentiation based on the key morphological descriptors for the 12 TGMS lines.

	Lines					
Traits	DRR-9S	DRR-2S	IR70978-22S	DRR-1S	IR73827-23S	DRR-5S
Panicle type Stigma colour	Partially open Yellow	Compact White	Compact Yellow	Partially open Purple	Partially open Purple	Partially open White
Groups	I	2	3	4		5
Apiculus pigmentation Awning				Present Awnless	Absent Awnless	Absent Awnless
Subgroups				4-1	4-2	5-1
	Lines					
Traits	IR73834-21S	UPRI-95-140S	UPRI-95-167S	DRR-4S	DRR-7S	DRR-3S
Panicle type Stigma colour	Partially open White	Open Purple	Open Purple	Compact Purple	Compact Purple	Compact Purple
Groups	5	6			7	
Apiculus pigmentation Awning	Absent Awned	Present Awnless	Present Awned	Absent Awnless	Absent Awned	Present Awnless
Subgroups	5-2	6-1	6-2	7-1	7-2	7-3

TGMS lines). The average GS value within the lines developed at DRR, IRRI and GBPAUT were 0.53, 0.71 and 0.78, respectively. The average GS values between the DRR and IRRI Lines, IRRI and GBPAUT lines and DRR and GBPAUT lines were 0.62, 0.74 and 0.65, respectively. UPGMA cluster analysis was performed using Euclidian distance matrix (1 – MD = MS), to generate a dendrogram (figure 1a). The similarity coefficient values ranged from 0.54 to 0.89 with a good fit to MS matrix (r_{cs} = 0.74; P = 0.005). All genotypes were grouped into two major clusters at 0.28% similarity. The first major cluster was divided into two subclusters consisting of the two GBPAUT lines, (UPRI-95-140S and UPRI-95-167S), and one each of IRRI line (IR73827-23S) and DRR (DRR-3S) in one cluster and one TGMS line of (DRR-1S) formed another cluster. The second major cluster consisted of DRR lines and two IRRI Lines. In PCA (figure 1b), the first two components having eigen values > 1 explained about 57% of the total variation. The PCA clustered the TGMS lines of GBPUAT (UPRI-95-140S and UPRI-95-167S) in one cluster, while the three DRR lines, namely DRR-4S, DRR-5S and DRR-7S were found to be in another cluster. Remaining lines of DRR and IRRI were scattered in the component and were clearly isolated from the lines of GBPAUT.

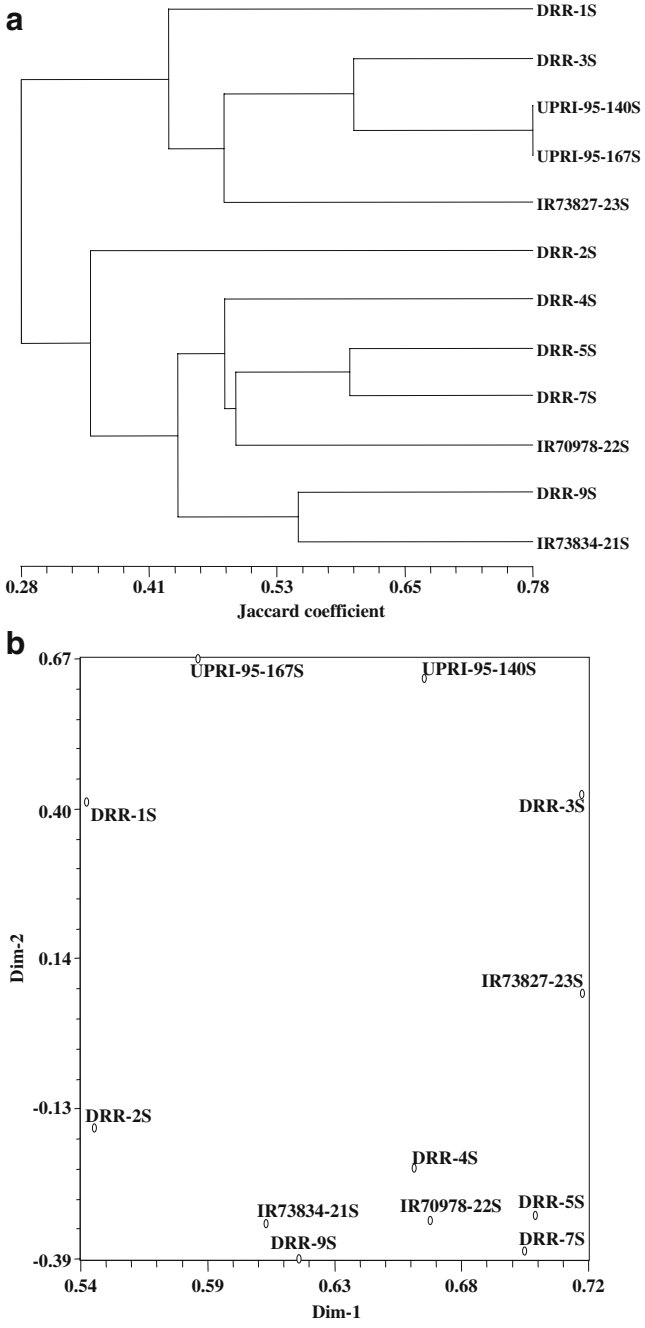

Figure 1. (a) Dendrogram showing genetic relationship among the 12 TGMS lines on the basis of morphological traits. (b) Principal component analysis (PCA) of 12 TGMS lines based on morphological traits.

Genetic similarity and cluster performance on the basis of SSR marker analysis

A total of 83 alleles were amplified using 30 SSR markers out of which 27 were polymorphic. About 2–6 polymorphic alleles were amplified per primer pair and the average number of alleles per primer pair was 2.76. Three primer pairs were found to be monomorphic (RM119, RM167 and RM161) and were not considered for further analysis. The primer pair RM70 amplified six alleles, while RM276 generated five polymorphic alleles followed by RM336, RM424 and RM335, each of which amplified four polymorphic alleles, RM136 and RM144, each amplified three polymorphic alleles (figure 2) and each of the remaining SSR markers generated two polymorphic alleles. The total number of alleles and the PIC values of the markers amplified among the TGMS lines are presented in table 4.

The average genetic similarity among the TGMS lines ranged from 0.43 to 0.94. The average genetic similarity within the DRR, IRRI and GBPAUT lines were 0.45,

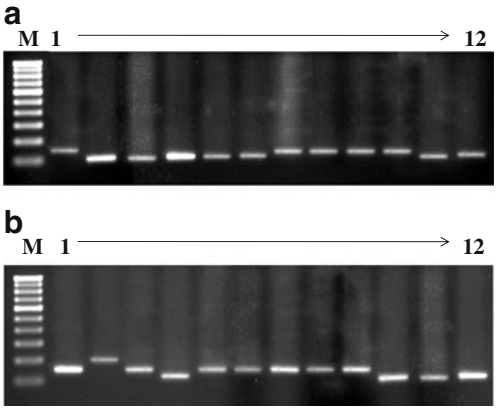

Figure 2. Amplification pattern of TGMS lines using marker (a) RM136 and (b) RM144; M, ladder 50 bp and lanes 1, DRR-1S; 2, DRR-2S; 3, DRR-3S; 4, DRR-4S; 5, DRR-5S; 6, DRR-7S; 7, DRR-9S; 8, IR70978-22S; 9, IR73827-23S; 10, IR73834-21S; 11, UPRI-95-140S and 12, UPRI-95-167S.

Table 4. Allelic distributions and PIC values of 30 SSR markers among 12 TGMS lines.

SSR markers	No. of alleles	Observed PIC values
RM119	2	0.75
RM127	2	0.58
RM154	2	0.87
RM164	2	0.84
RM101	2	0.82
RM111	2	0.64
RM136	3	0.94
RM144	3	0.79
RM149	3	0.90
RM167	1	0.00
RM168	2	0.86
RM177	3	0.88
RM160	2	0.58
RM161	1	0.00
RM169	3	0.89
RM153	2	0.64
RM128	2	0.82
RM163	3	0.92
RM190	3	0.95
RM592	3	0.91
RM336	4	0.91
RM335	4	0.95
RM70	6	0.97
RM204	3	0.89
RM276	5	0.95
RM418	3	0.89
RM424	4	0.95
RM535	2	0.75
RM287	3	0.93
RM499	2	0.70

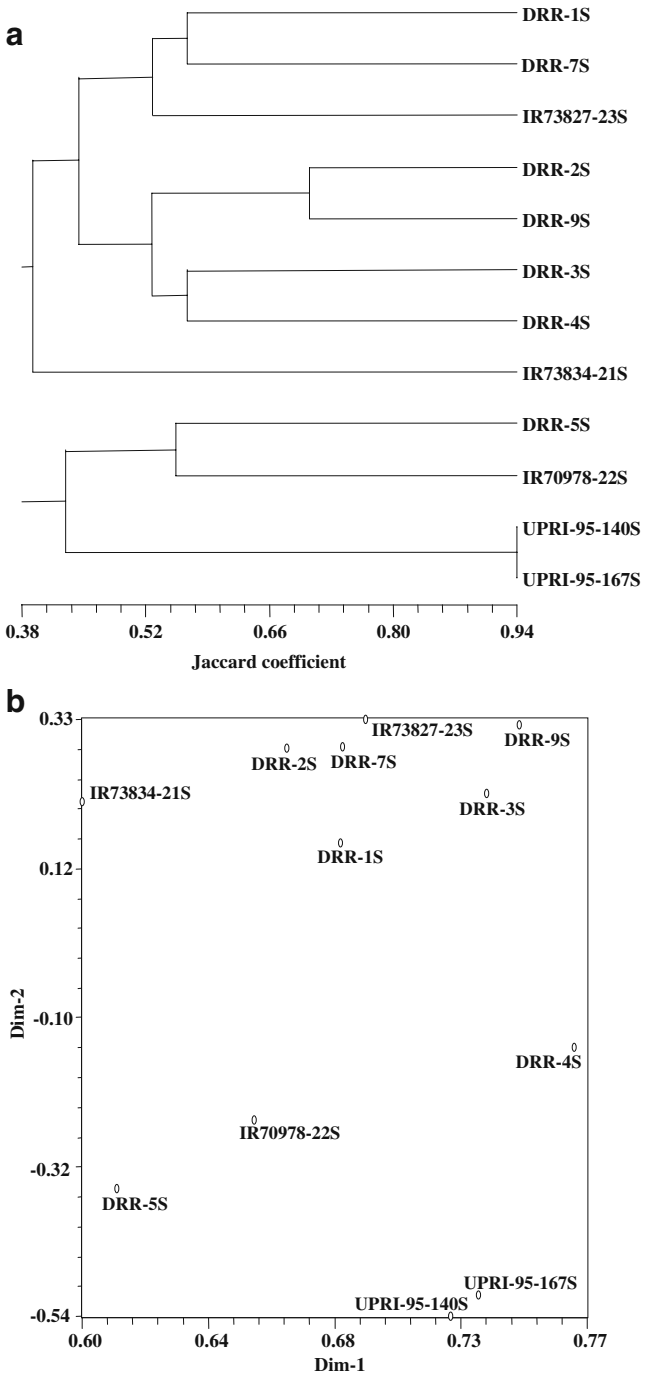

Figure 3. (a) Dendrogram showing genetic relationship among the 12 TGMS lines on the basis of SSR data. (b) Principal component analysis (PCA) of 12 TGMS lines based on SSR data.

0.44 and 0.94, respectively. The average genetic similarity between the DRR and IRRI lines, IRRI and GBPAUT lines and DRR and GBPAUT lines were 0.40, 0.36 and 0.42, respectively. UPGMA cluster analysis was performed using Jaccard's (1901) similarity coefficient matrix calculated from SSR markers to generate a dendrogram for the 12 TGMS lines (figure 3a). The similarity coefficient ranged from 0.38 to 0.94, with a good fit to GS matrix ($r_{cs} = 0.82$). All lines were grouped into two major clusters at 38% similarity coefficient cut-off point. The first cluster was divided into three subclusters at 40% similarity. These consisted of two DRR lines in the first subcluster (DRR-1S and DRR-7S) and one IRRI line in the second subcluster (IR73827-23S) and only the DRR lines in the third subcluster. The IRRI line IR73834-21S was well separated from other lines. The second cluster again consisted of two subclusters. The first subcluster consisted of one DRR line (DRR-5S) and one IRRI line (IR70978-22S). The second subcluster consisted of two TGMS lines of GBPAUT origin (UPRI-95-140S and UPRI-95-167S), which showed 94% genetic similarity and grouped in one cluster perhaps because they are sister lines. In the PCA (figure 3b), the first four components explained about 87% of the variation having eigen value >1. The first two axes explained 58% of the variation and could clearly separate all the TGMS lines according to their origin. The first cluster comprised majority of the DRR lines (DRR-2S, DRR-1S, DRR-7S, DRR-9S and DRR-3S) and IR73827-23S. The second cluster consisted of two TGMS lines, UPRI-95-140S and UPRI-95-167S. Two lines of each DRR (DRR-4S and DRR-5S) and IRRI (IR70978-22S and IR73834-21S) lines were not part of any major cluster.

Table 5. Genetic similarity between and within the group of TGMS lines on the basis of morphological and SSR data.

	Genetic similarity				
	Between the groups			Within the groups	
Groups	DRR lines	IRRI lines	GBPAUT lines	Molecular similarity	Morphological similarity
DRR lines		0.40	0.42	0.45	0.53
IRRI lines	**0.62**		0.36	0.44	0.71
GBPAUT lines	**0.65**	**0.74**		0.94	0.78
Average genetic similarity				0.43	0.54

Bold figures indicate morphological similarities within the groups.

Comparison of data derived from SSR marker analysis and morphological traits

Genetic similarity between and within the groups of TGMS lines on the basis of morphological and SSR data is presented in table 5. The correlation between morphological similarity matrix (MS = 1 – MD) and molecular similarity matrix (GS) were not significant and very low (Mantel test, $r = 0.06$; $P = 0.69$). The mantel Z-test statistic was significant between MS and GS matrices ($Z = 4.47$, $P < 0.0001$) as well as between the cophenetic matrices of GS and MS ($Z_c = 4.85$, $P < 0.0001$) indicating a good correspondence between the two similarity measures. There is some degree of correspondence between dendrograms generated by morphological and molecular data wherein the lines developed at DRR and GBPUAT were differentiated, while the lines developed at IRRI could not be separated from those developed at DRR and GBPUAT.

Estimation of hybrid performance

The plant yield of the parents ranged from 12.25 g (UPRI-90-167S) to 25.25 g (BCW-56) with an average of 18.63 g, while the hybrid ranged from 19.29 g (UPRI-90-167S/BCW-56) to 32.25 g (IR73827-23S/Shalivahana) with an average of 26.59 g (table 6). TGMS lines DRR-1S and IR73827-23S from cluster 1 (as shown in table 6) showed similar heterotic performance when crossed with three common pollinators (Shalivahana, BCW-56 and JGL-384) with the average yield of 28.74 g. While, the TGMS lines UPRI-90-140S and UPRI-90-167S from cluster 2 (as shown in table 6) showed similar heterotic performance when crossed with the same pollinators, indicating that the genotypes clustered in a cluster bear similar heterotic potential as compared to the genotypes falling under different clusters.

Discussion

The two-line-breeding system of hybrid rice is considered as improvement over three-line-breeding system, because there is no need to restore the fertility as compared to the three line breeding system. Hence, there is more choice of crossing the diverse parents leading to better heterosis in the hybrids (Xiao *et al.* 1996). Molecular markers offer the opportunity to assess variation at the DNA sequence level and have become an important tool in plant genetics (Gebhardt *et al.* 1991). Microsatellite markers have been used in rice for assessing genetic diversity (Subudhi *et al.*

Table 6. Comparison of yield performance of hybrids based on the clustering pattern of TGMS lines.

	Pollinator parents			
TGMS line	Shalivahana (23.20)	BCW-56 (25.35)	JGL-384 (19.87)	Mean±SD
Cluster-1				
DRR-1S (16.15)	28.00	26.26	30.62	28.29±2.19
IR73827-23S (18.25)	32.25	28.10	27.23	29.19±2.68
			Average	28.74
Cluster-2				
UPRI-90-140S (15.20)	26.00	25.37	25.25	25.54±0.40
UPRI-90-167S(12.45)	25.82	19.29	25.00	23.37±3.56
			Average	24.45

Number in parenthesis indicates yield of parents in grams; SD, standard deviation.

1997; Aggarwal *et al.* 1999; Garland *et al.* 1999; Virk *et al.* 2000; Ravi *et al.* 2003; Yu *et al.* 2003) and also genetic purity of genotypes (Olufowote *et al.* 1997; Yashitol *et al.* 2002). In the present study, we opted for utilization of 30 uniformly distributed EST and nonEST derived SSR markers for the characterization of 12 TGMS lines derived from three different sources (DRR, IRRI and GBPUAT).

Out of the 30 SSR primer pairs analysed, 27 were found to be polymorphic and generated a total of 83 alleles. The average number of alleles per primer was 2.76. This value is lower than the value of 4.3 obtained by Davierwala *et al.* (2000) since, the average number of alleles were considerably lower in EST-SSRs as compared to nonEST SSRs. Gupta *et al.* (2003) has also reported lower level of polymorphism with respect to EST-SSRs. However an important feature of EST-SSRs is that these are a part of expressed sequences of the rice genome and hence represent functional variability as compared to genomic SSRs, which may essentially not be associated with any trait of interest. The PIC values obtained from 30 SSR primer pairs ranged from 0.54 (RM160) to 0.96 (RM70) and the average value of PIC (0.82) was lower than the PIC value (0.89) obtained by Panaud *et al.* (1996). This could be because the present investigation involved a high proportion of closely related cultivars, resulting in the lower PIC values. Utilization of hypervariable/hyperpolymorphic SSR markers could have contributed to the enhancement of PIC values (Struss and Plieske 1998).

The microsatellite markers discriminated between the DRR and GBPUAT accessions. However, IRRI accessions could not be clearly distinguished either by morphological or molecular analysis. Both the GBPUAT lines viz., UPRI-95-140S and UPRI-95-167S showed 94% similarity, as both the genotypes were sister lines by pedigree. UPGMA cluster analysis of SSRs based on similarity matrix using Jaccard's similarity coefficient clearly distinguished a majority of the DRR lines from IRRI and GBPUAT lines. Four DRR lines (DRR-2S, DRR-3S, DRR-4S and DRR-9S) form the same group based on molecular and morphological similarity coefficients.

Low correlation (Mantel test $r = 0.06$; $P = 0.69$) was observed between the similarity matrices (GS) generated using SSRs and morphological data. Similar correlation ($r = 0.25$, $P < 10^{-5}$) pattern were also reported between Tunisian winter barley using SSR and morphological traits (Hamza *et al.* 2004). The very low relationship revealed by morphological and molecular markers could also be due to sampling deviation and failure of phenotypes to differentiate the genotypes precisely (Bohn *et al.* 1999; Dreisigacker *et al.* 2004). Sampling effects due to inappropriate genome coverage might have increased the standard error in estimating genetic similarity using molecular markers resulting in poor correlation between morphological and molecular genetic similarity studied by Bohn *et al.* (1999).

Genetic diversity is fundamentally important for developing heterotic rice hybrids. Grouping based on SSR markers, in general, agreed with the parental pedigree information provides indispensable information regarding the genetic diversity among the genotypes. Varieties and lines sharing the common ancestry were clustered in to the same group, indicating the efficiency of SSR markers in detecting the genetic diversity in rice. The information derived from the present study regarding genetic diversity of parental lines and its importance in identifying the genotypes clustering into same heterotic pools were consistent with the conclusions given by studies of Xangsayasane *et al.* (2010). Marker based genetic distance along with the morphological characterization forms a reliable tool in determining genetic relationships. Genotypes having similar genetic relationships clustered in the same group possess similar heterotic behaviour.

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