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RESEARCH ARTICLE

Prediction of heterosis in rice based on divergence of morphological and molecular markers

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Abstract. Identifying the best performing hybrid without a field test was essential to save resources and time. In this study, the genetic divergence was estimated using morphological and expressed sequence tag (EST)-derived simple sequence repeats (SSR) markers. Cluster analysis showed that APMS6A and RPHR 1005 belong to groups I and II, respectively, and the hybrid combination recorded the highest mean grain yield of 32.25 g among generated 40 F_1s with standard heterosis of 8.4% over hybrid check, KRH2. The coefficient of marker polymorphism (CMP) value was calculated based on EST-SSR markers; it ranged from 0.40 to 0.80, and a higher CMP value of 0.80 was obtained for the parental combination APMS6A × RPHR1005. We predicted heterosis for 40 F_1s based on correlation between CMP and standard heterosis in different traits with standard varietal and hybrid checks indicating positive correlation and significant value for grain yield per plant ($r = 0.58$**), productivity per day ($r = 0.54$**), productive tillers ($r = 0.34$*) and panicle weight ($r = 0.42$**). This study revealed that the relationship of molecular marker heterozygosity, along with the combining ability, high mean value of different traits, grouping of parental lines based on morphological and molecular characterization is helpful to identify heterotic patterns in rice.

Keywords. diversity analysis; correlation; coefficient of marker polymorphism; prediction; heterosis.

Introduction

World population is expected to reach 8.5 billion by 2030 (UN DESA 2016) and needs food to keep pace with the growing population. Rice is one of the most important and staple food for more than a half of the global population. In India, population is expected to reach 1.7 billion by 2030 and it is necessary to produce 156 metric ton rice (vision 2030, ICAR). Further, increased yield has to be achieved with less resources and subcondition environment. Among the available options, hybrid technology is one which gives a higher yield of about 20–25% over inbred varieties (Yuan *et al.* 1989). Heterosis is a phenomenon and is exploited in many crops including rice, which leads to increased global production substantially. In rice, hybrid vigour was first reported by Jones (1926). In 1976, China exploited heterosis in rice. Later, India released many rice hybrids and cultivated rice hybrids in farmers' fields using a three-line system involving a cytoplasmic male sterile line, maintainer line and restorer line.

The three-line system of hybrid rice breeding requires a lot of effort, field evaluation, skilled manpower and is time consuming. To overcome this herculean task, identification of the best parental combination without field trial gains importance for increasing the breeding efficiency (Hallauer *et al.* 1988). In maize, prediction of heterosis between inbreds was done based on various parameters such as morphological characters, pedigree data, physiological parameters and biochemical and isozyme analysis (Smith and Smith 1989; Smith *et al.* 1990). However, measuring heterosis in terms of yield is more accurate in rice though a complex phenomenon (Zhang *et al.* 2010).

With the advent of molecular techniques, the efficiency of plant breeding has been significantly increased (Tanksley *et al.* 1992). Among the molecular markers, simple sequence repeats (SSRs) are more reliable and

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practical with high-genome coverage (Stuber *et al*. 1992). Expressed sequence tag-simple sequence repeats (EST-SSRs) are another class of SSR markers useful in detection of genetic dissimilarities. The F_1 heterosis expression is known to be related to genetic distance between the parents (Lee *et al*. 1989; Smith *et al*. 1990; Stuber *et al*. 1992). However, most of the times it is not significantly correlated because the F_1 performance depends on the origin of species/genotypes used in the cross, use of lower number of markers, poor association between marker and gene/quantitative trait loci (QTLs) with heterosis, heterosis measurement in terms of complex traits such as yield, interaction among genes/QTLs, epigenetic effects, sampling error and environmental interaction with character of study (Melchinger *et al.* 1990, 1998). Recently, genomewide prediction of heterosis has been attempted with the help of molecular markers by many workers in rice and it has been reported that with appropriate analytical methods, the prediction of heterosis accuracy can be improved (Wang *et al*. 2016; Jaikishan *et al*. 2010). The objective of this study was to determine the genetic divergence among the parental lines through morphological and molecular markers and to calculate correlation coefficients of marker polymorphism based on EST-SSRs and standard heterosis for grain yield and other yield-contributing traits in hybrids to predict yield heterosis.

Materials and methods

Eight restorers were crossed with five CMS lines in line × tester mating design to obtain 40 F_1 hybrids (table 1) at Rajendranagar research farm, ICAR-Indian Institute of Rice Research, Hyderabad. The seeds of the F_1 and their parents were placed in Petri dishes for germination and the germinated seedlings were transferred to small raised beds. Seedlings of 28 days were transplanted in randomized complete block design with three replications in main field. All recommended package of practices were followed to raise healthy crop. Observations were recorded for 12 yield and yield-related characters, namely days to 50% flowering (DFF), days to maturity, plant height (cm), number of productive tillers per plant, number of unproductive tillers per plant, panicle length (cm), panicle weight (g), number of filled grains per panicle, spikelet fertility (%), 1000 seed weight (g), grain yield per plant (g) and per day productivity (kg/ha) on parents and hybrids (tables 2 and 3).

Molecular analysis

DNA was extracted using a modified method from the protocol of Zhang *et al*. (1995). Twenty-five EST-derived SSR markers (table 4) distributed across 12 chromosomes were selected for genetic diversity analysis available at http://gramene.org. Polymorphism between genotypes was studied following polymerase chain reaction (PCR) conditions described by Chen *et al.* (1997) with modifications. The PCR machine was programmed with initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were resolved on 3.0% agarose gel in 0.5× TBE buffer in a mid-size electrophoresis unit for 3 h at 75 V, stained with ethidium bromide (0.5 μg/mL) and photographed using UV light.

The polymorphic bands are scored manually for the presence '1' and absence '0' for each genotype. The polymorphism information content (PIC) for each EST-SSR marker was calculated using online software 'Polymorphism Information Content Calculator' available at https://www.liverport.ac.uk/~kempsj/pic.html.

Similarity, a matrix was generated by binary marker data using the SimQual program NTSYS-pc software v. 2.02 (Rohlf 1998) for calculating genetic distance of genotypes by Jacord similarity coefficient and dendrogram constructed using similarity coefficients by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) (Mathew *et al*. 2000) method.

Data analysis

Genetic divergence

Mean value of 12 characters were used to study the genetic diversity between genotypes using D^2 statistics given by Mahalanobis (1936). Based on D values (inter-cluster distance), a cluster diagram was prepared using the scale given by Rao (1952).

The D^2 value between the ith and jth genotypes for 'p' character was calculated as:

$$D_{ij}^2 = \sum_{t}^{p} (y_{it} - y_{jt})^2,$$

where y_{it}, uncorrelated mean value of the ith genotype for t character; y_{jt}, uncorrelated mean value of the jth genotype for t character; D_{ij}^2, D^2 between the ith and jth genotype.

Estimation of heterosis

Heterosis was estimated using the following formula:

$$\text{Standard heterosis}(\%) = \frac{F_1 - \text{Mean of check}}{\text{Mean of check}} \times 100$$

To test the significance for heterosis, standard error (SEm) is calculated using the following formula:

$$\text{SEm} = \sqrt{2\sigma^2 e/r}$$

where $\sigma^2 e$ is the error mean square and r is the number of replications.

Table 1. Mean performance of the lines, testers, hybrids and checks for the given morphological characters.

	Genotypes	DFF	DTM	PH (cm)	No. of PT	No. of UPT	PL (cm)
Lines							
1	APMS 6A	104	135.00	94.15	17.55	2.73	24.58
2	PMS 10A	109	141.00	96.23	19.25	2.69	24.87
3	PUSA 5A	104.5	126.50	88.85	19.25	3.62	23.92
4	IR 68897A	94.5	126.50	82.60	17.80	6.39	25.38
5	IR 58025A	101.5	131.00	91.67	16.43	10.72	25.25
	CMS lines mean	102.7	132	90.70	18.06	5.23	24.80
Testers							
6	RPHR 1004	102.5	131.00	95.98	17.15	5.70	23.75
7	RPHR 1005	104.5	134.00	97.75	15.20	6.75	22.55
8	RPHR 1096	110.5	141.50	101.42	16.15	1.22	23.21
9	BCW 56	111	139.00	111.42	15.90	1.48	24.11
10	KMR 3R	109	139.00	106.08	17.75	0.97	23.36
11	EPLT 109	107	135.50	101.65	15.90	1.49	25.45
12	SG-22-289-3	105.5	135.50	104.05	14.80	1.84	24.42
13	RPHR 619-2	105.5	134.50	102.50	18.75	2.80	23.61
	Restorer mean	106.94	136.25	102.60	16.45	2.78	23.80
	Parental mean	105.12	134.43	97.50	17.13	3.82	24.23
Hybrids							
1	APMS6A × RPHR1004	100.5	131.00	98.32	18.88	2.10	25.44
2	APMS6A × RPHR1005	101	132.00	105.05	20.80	2.15	27.36
3	APMS6A × RPHR1096	102.5	134.50	125.75	18.02	3.94	27.01
4	APMS6A × BCW56	99	132.00	119.00	20.77	1.73	26.03
5	APMS6A × KMR3R	100	132.00	111.17	23.75	2.07	26.96
6	APMS6A × EPLT109	102.5	135.50	102.15	19.65	0.91	27.59
7	APMS6A × SG-22-289-3	100	131.00	106.10	18.80	9.53	25.67
8	APMS6A × RPHR619-2	103.5	134.50	97.85	19.55	2.74	26.11
9	PMS10A × RPHR1004	104.5	135.50	96.67	17.85	2.17	26.70
10	PMS10A × RPHR1005	105.5	135.50	100.42	22.17	1.34	27.55
11	PMS10A × RPHR1096	105.5	136.50	101.25	20.00	1.77	26.22
12	PMS10A × BCW56	103	133.50	111.25	19.75	1.72	25.88
13	PMS10A × KMR3R	110.5	141.00	106.08	17.50	2.51	26.34
14	PMS10A × EPLT109	105	135.00	109.65	21.85	3.24	27.36
15	PMS10A × SG-22-289-3	103	133.50	117.05	19.17	1.57	25.97
16	PMS10A × RPHR619-2	103	133.50	111.30	17.90	6.17	27.00
17	PUSA5A × RPHR1004	101.5	132.00	97.58	15.98	14.40	25.11
18	PUSA5A × RPHR1005	100	130.50	101.00	17.68	1.06	26.64
19	PUSA5A × RPHR1096	101.5	132.50	113.40	16.70	4.27	26.38
20	PUSA5A × BCW56	100.5	131.00	116.10	20.00	2.00	26.95
21	PUSA5A × KMR3R	99.5	131.00	103.50	18.19	0.97	26.89
22	PUSA5A × EPLT109	102.5	133.00	101.65	21.02	1.51	27.21
23	PUSA5A × SG-22-289-3	99	129.00	98.50	18.68	2.34	26.14
24	PUSA5A × RPHR619-2	101	131.00	101.50	17.85	4.15	26.63
25	IR68897A × RPHR1004	94.5	125.00	98.83	19.90	3.10	26.04
26	IR68897A × RPHR1005	95.5	125.50	101.45	22.20	2.81	26.05
27	IR68897A × RPHR1096	98	129.00	106.65	20.40	2.65	26.62
28	IR68897A × BCW56	99	130.00	120.80	17.80	2.37	26.86
29	IR68897A × KMR3R	99	129.00	101.35	17.93	2.14	27.05
30	IR68897A × EPLT109	98.5	129.00	97.50	16.65	2.15	26.27
31	IR68897A × SG-22-289-3	96.5	128.00	98.55	16.50	4.05	26.23
32	IR68897A × RPHR619-2	97	127.00	106.30	18.80	2.15	25.62
33	IR58025A × RPHR1004	101.5	131.50	98.42	16.90	2.43	26.67
34	IR58025A × RPHR1005	103.5	134.50	100.27	21.55	5.35	27.01
35	IR58025A × RPHR1096	102.5	133.50	97.50	17.08	2.90	25.53
36	IR58025A × BCW56	100.5	131.00	104.10	20.83	2.17	29.00
37	IR58025A × KMR3R	102.5	132.00	101.55	22.25	2.75	27.05
38	IR58025A × EPLT109	109	139.00	95.65	20.00	1.80	26.38
39	IR58025A × SG22-289-3	99	129.00	102.65	17.93	3.17	26.45
40	IR58025A × RPHR619-2	101.5	132.00	100.15	19.75	4.41	26.68

Table 1 (*contd*)

	Genotypes	DFF	DTM	PH (cm)	No. of PT	No. of UPT	PL (cm)
	Crosses mean	101.31	132.04	104.6	19.22	3.07	26.56
	Grand mean	102.43	132.75	102.66	18.61	3.27	25.89
Checks							
1	KRH 2	105.5	135.50	96.00	22.15	2.90	27.75
2	Jaya	102.5	133.00	89.55	26.00	1.60	25.30
Lines							
1	APMS 6A	3.01	157.05	80.24	18.75	19.60	0.15
2	PMS 10A	2.39	100.65	80.78	21.50	22.25	0.16
3	PUSA 5A	2.57	118.75	85.37	23.00	21.75	0.17
4	IR 68897A	3.57	126	77.27	21.50	19.05	0.15
5	IR 58025A	3.93	155.75	82.24	20.50	18.40	0.14
	CMS lines mean	3.09	131.64	81.18	21.05	20.21	0.15
Testers							
6	RPHR 1004	3.20	135.06	76.86	18.00	18.75	0.14
7	RPHR 1005	3.34	166.05	80.34	19.50	21.05	0.16
8	RPHR 1096	3.95	144.7	76.84	20.50	20.75	0.15
9	BCW 56	3.73	133.45	71.43	21.50	19.25	0.14
10	KMR 3R	3.89	154.2	77.84	19.50	21.50	0.15
11	EPLT109	4.30	143.2	70.89	21.50	19.00	0.14
12	SG-22-289-3	2.67	112.1	63.93	18.50	20.50	0.15
13	RPHR 619-2	3.66	138.05	76.66	21.50	20.75	0.15
	Restorer mean	3.59	140.85	74.34	20.06	20.19	0.15
	Parental mean	3.38	136.90	77.27	20.48	20.20	0.15
Hybrids							
1	APMS6A × RPHR1004	3.99	178.1	74.03	19.00	25.00	0.19
2	APMS6A × RPHR1005	4.37	195.6	76.62	18.50	32.25	0.24
3	APMS6A × RPHR1096	4.73	176.05	80.86	21.50	25.50	0.19
4	APMS6A × BCW56	4.51	164.45	72.29	23.00	23.00	0.17
5	APMS6A × KMR3R	4.98	194.65	85.43	20.50	30.00	0.23
6	APMS6A × EPLT109	5.12	194.1	84.82	21.00	29.00	0.21
7	APMS6A × SG-22-289-3	3.29	147.05	67.47	19.50	21.60	0.16
8	APMS6A × RPHR619-2	4.67	184.2	74.48	22.50	26.40	0.20
9	PMS10A × RPHR1004	4.38	169.35	77.94	19.50	25.40	0.19
10	PMS10A × RPHR1005	4.75	204.35	83.52	18.50	31.55	0.23
11	PMS10A × RPHR1096	4.56	174.65	77.76	21.50	26.20	0.19
12	PMS10A × BCW56	5.02	197.6	75.97	24.50	27.15	0.20
13	PMS10A × KMR3R	4.14	179.45	69.33	21.50	25.10	0.18
14	PMS10A × EPLT109	5.40	194.75	82.96	23.50	29.25	0.22
15	PMS10A×SG-22-289-3	3.93	160.55	76.98	19.00	23.90	0.18
16	PMS10A × RPHR619-2	3.95	147.65	69.55	21.50	25.60	0.19
17	PUSA5A × RPHR1004	3.58	155.8	72.23	19.50	18.35	0.14
18	PUSA5A × RPHR1005	4.01	156.45	70.85	20.00	25.80	0.20
19	PUSA5A × RPHR1096	4.35	169	75.85	21.00	22.25	0.17
20	PUSA5A × BCW56	4.67	174.7	72.27	23.50	25.75	0.20
21	PUSA5A × KMR3R	4.77	194.2	79.03	20.50	25.50	0.19
22	PUSA5A × EPLT109	5.42	191.37	79.68	23.00	29.75	0.22
23	PUSA5A × SG-22-289-3	4.27	166.1	65.62	23.50	21.25	0.16
24	PUSA5A × RPHR619-2	4.04	162.55	72.85	20.50	23.15	0.18
25	IR68897A × RPHR1004	4.45	198.55	78.85	21.00	27.00	0.22
26	IR68897A × RPHR1005	3.54	130.4	61.00	21.00	22.25	0.18
27	IR68897A × RPHR1096	4.38	214.2	80.96	18.50	26.65	0.21
28	IR68897A × BCW56	4.24	139	66.81	23.00	22.60	0.17
29	IR68897A × KMR3R	4.16	170.77	75.79	18.00	26.65	0.21
30	IR68897A × EPLT109	4.71	183.05	76.56	21.50	25.75	0.20
31	IR68897A × SG-22-289-3	4.66	161.15	74.31	25.00	20.00	0.16
32	IR68897A × RPHR619-2	5.18	184	81.50	22.00	26.60	0.21
33	IR58025A × RPHR1004	4.12	159.7	77.05	18.00	25.25	0.19
34	IR58025A × RPHR1005	4.94	191.4	83.38	21.50	28.10	0.21
35	IR58025A × RPHR1096	3.94	166.2	70.74	19.50	24.75	0.19
36	IR58025A × BCW56	5.11	182.4	73.98	22.00	31.70	0.24
37	IR58025A × KMR3R	4.34	171.7	72.26	20.50	28.00	0.21
38	IR58025A × EPLT109	4.85	167.8	76.38	22.50	26.90	0.19

Table 1 (*contd*)

	Genotypes	DFF	DTM	PH (cm)	No. of PT	No. of UPT	PL (cm)
39	IR58025A × SG22-289-3	3.97	174.3	67.61	20.50	22.10	0.17
40	IR58025A × RPHR619-2	4.81	185.9	77.82	23.00	24.90	0.19
	Crosses mean	4.45	175.33	75.33	21.1	25.70	0.19
	Grand mean	4.15	164.42	75.84	20.91	24.13	0.18
Checks							
1	KRH 2	4.70	205.5	85.38	21.50	29.75	0.22
2	Jaya	4.77	188.5	84.07	25.60	27.75	0.21

DFF, days to 50% flowering; DTM, days to maturity; PH, plant height; no. of PT, no. of productive tillers; no. of UPT, no. of unproductive tillers; PL, panicle length.

Table 2. List of rice lines used in the hybrid rice breeding programme.

Testers/males	Designation	Source	Pedigree
Restorer line 1	RPHR 1004	IIRR, TS	BPT5204 × SC5 126-3-2
Restorer line 2	RPHR 1005	IIRR, TS	BPT5204 × SC5 126-3-2
Restorer line 3	RPHR 1096	IIRR, TS	BR827-35×SC5 21-1-2-1-4
Restorer line 4	RPHR 619-2	IIRR, TS	BR827-35×SC5-23-3-4-2
Restorer line 5	BCW 56		IR58025A × (Jaya × IR40750)
Restorer line 6	KMR 3R	Mandya, KN	Jaya × IR29723-143
Restorer line 7	EPLT 109	IIRR, TS	Swarna × 9314
Restorer line 8	SG-22-289-3	IIRR, TS	Samba Mahsuri × CPSLO17
Lines/females			
CMS line 1	APMS 6A	Maruteru, AP	–
CMS line 2	PMS 10A	Pau, Kapurtala	–
CMS line 3	PUSA 5A	IARI, New Delhi	IR58025A × PUSA 150-21-1-1-2
CMS line 4	IR 68897A	IRRI	IR62829A × IR62856-15-3-1-1-7-5-10-3
CMS line 5	IR 58025A	IRRI	IR48483A × Pusa167-120-3-2
Variety checks			
Variety Check 2	Jaya	IIRR, TS	TN1 × T 141
Hybrid check			
HYBRID Check 1	KRH 2	Mandya, KN	IR58025A × KMR3R

Least significance difference (critical difference (CD)) for heterosis

The significant differences between two estimates of heterosis were tested by calculating least significant difference by multiplying the SEm with the appropriate Student's t value of the respective error degrees of freedom at the desired level of probability: CD = SEm × t table value at error degrees of freedom.

Prediction of heterosis

Correlating coefficient of marker polymorphism (CMP) based on EST-SSR markers with standard heterosis (Jaikishen *et al.* 2010) is given by

$$\text{Coefficient of marker polymorphism (CMP)} = \frac{\text{No. of polymorphic markers}}{\text{Total no. of markers}}.$$

Correlation

Correlation between CMP values and standard heterosis was calculated manually using Microsoft Excel Spreadsheet, 2007 version. The test of significance was estimated based on the specified probability value for the number of treatments.

Results

The experimental results are presented below.

Genetic divergence

The cluster analysis was performed using Mahalanobis Euclidean distance matrices calculated from the mean performances of 12 morphological characters (table 1). The 15 genotypes were grouped into four clusters based on the

Table 3. List of F_1 hybrids used for generating field data.

	Crosses		Crosses
1	APMS6-A×RPHR1004	21	PUSA5-A×KMR-3R
2	APMS6-A×RPHR1005	22	PUSA5-A×EPLT109
3	APMS6-A×RPHR1096	23	PUSA5-A×SG-22-289-3
4	APMS6-A×BCW56	24	PUSA5-A×RPHR619-2
5	APMS6-A×KMR-3R	25	IR68897-A×RPHR1004
6	APMS6-A×EPLT109	26	IR68897-A×RPHR1005
7	APMS6-A×SG-22-289-3	27	IR68897-A×RPHR1096
8	APMS6-A×RPHR619-2	28	IR68897-A×BCW56
9	PMS10-A×RPHR1004	29	IR68897-A×KMR-3R
10	PMS10-A×RPHR1005	30	IR68897-A×EPLT109
11	PMS10-A×RPHR1096	31	IR68897-A×SG-22-289-3
12	PMS10-A×RPHRBCW56	32	IR68897-A×RPHR619-2
13	PMS10-A×KMR-3R	33	IR58025-A×RPHR1004
14	PMS10-A×EPLT109	34	IR58025-A×RPHR1005
15	PMS10-A×SG-22-289-3	35	IR58025-A×RPHR1096
16	PMS10-A×RPHR619-2	36	IR58025-A×BCW56
17	PUSA5-A×RPHR1004	37	IR58025-A×KMR-3R
18	PUSA5-A×RPHR1005	38	IR58025-A×EPLT109
19	PUSA5-A×RPHR1096	39	IR58025-A×SG-22-289-3
20	PUSA5-A×BCW56	40	IR58025-A×RPHR619-2

Table 4. List of EST-derived SSR markers used for molecular characterization.

	Chr. no.	Name of marker	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
1	1	RMES 1-2	CAGCGTGATCAACCTGAAGA	CACGAATCAGAG CACAAACG
2	1	UGRM201	GGGGACGAGTGGTGCTGT	CGTGTGACGGCTCTGGTG
3	1	RMES 1-1	CAGTTTGGTTTGGATTGGCT	TTAGGGAAACAGCGATCACA
4	2	RMES 2-2	CACCTCCCAATCTTAACCCA	GGGAAGGTGTTGGAGGTGTA
5	3	RMES 3-2	ACGGATTCACTGGGTTCTGT	CACCAGAAAGCATCACCTCA
6	3	RMES 3-1	GCAAGGAAGAAACCAGCGTA	TTGTGGGCAAAAACAAATGA
7	4	RMES 4-1	ACACCCCTCAGCTTGTGAAG	TCATCCTTCGCCATCTAACC
8	4	RMES 4-2	CATCGAGGAGCAGCTATGGT	CCTTGAGCGAGTCCTTGAAC
9	5	ESSR Chr520.9	GTCACTCACCTCACCTGCCT	TGGGGGTCCTCCTCTTAGTT
10	6	RMES 6-1	CTGCCACCGGTGTAGCTAGT	TGGCCCCATCGTATATGAAC
11	6	RMES 6-2	GGAGGAAGATCACTCCAGCA	ACCTTATCAGCAACGCTTCG
12	6	ESSR Chr 6 26.5	GCAAGTGTGTTCTTCTTTCTTTTG	TCTTCCCATTTGGCTGAATC
13	7	RMES 7-1	CCTCTCTCCCTCCTGTCCTC	CCACGTAGGAGGCAAACATC
14	7	RMES 7-2	TGGCCCTCATGAGACATACA	TTAAGCAATCAAAGGGGGTG
15	8	RMES 8-1	GGAGGAGGAGGAGGATCTTG	CTTCTCCGACGACGAGTTCT
16	8	RMES 8-2	GGATCGATGACGGACAGAAT	GAGATGAGATTGCAACACGG
17	9	RMES 9-1	CTGGAGATCGATCGAGAGGT	TAGCCAAGGAGACTGCCTGT
18	9	RMES 9-2	CCACGTTGATAAGCTCATTGC	TGGGCACCGAAAATAAAATC
19	10	RMES 10-1	CAGGGGGTACGTCTACAAGG	TATACCCGGCGAAATACGTC
20	10	RMES 10-2	AACAGTTCCAACAGAACCCG	TTATTTGCTGATGCTGCGAC
21	11	RMES 11-1	TTGACCTAGCCAACCTCACC	CGGAGTACACGTCCACCAC
22	11	RMES 11-2	GGTGGACTACTGCAGCACTG	TCTTGGTCGGGAACAGCTCC
23	12	ESSR Chr 12 23.4	TAGAAACTTCTGGAAGCGGC	AGAGGATGTGGAGGTCGATG
24	12	RMES 12-2	TGTGTTCATCACTGGCTTCG	CGTGTTGCAGAAGTTGCAGT
25	12	UGRM 785	TTTGACCTGACTAAATGGA	TTACAGAATCACAAGGAGAA

D^2 values. Of the four clusters formed, cluster II was the largest comprising five genotypes followed by cluster I with four genotypes, clusters III and IV each containing three genotypes (figure 1; tables 5 and 6).

The highest intercluster divergence occurred between clusters II and III (3202.41) followed by clusters I and II (1561.76) and clusters III and IV (1318.18), while the lowest divergence was noticed between clusters I and IV (985.74). The highest intracluster distance occurred in cluster IV (861.47) followed by clusters I (288.21) and II (198.11), while 'no' intracluster distance was observed in cluster III.

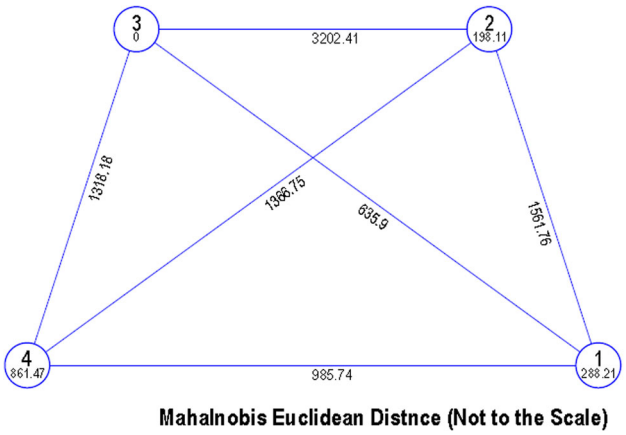


Figure 1. Intercluster and intracluster distances among the 15 genotypes for 12 morphological characters by Mahalanobis Euclidean distance.

Genetic diversity among parental lines using EST-derived SSR markers

Of the 25 markers used (table 4), 23 were found to be polymorphic and generated total of 375 alleles. The number of alleles per EST-SSR marker ranged from 1 to 7 with an average of 3.84. The marker RMES3-2 generated a maximum of seven alleles. The PIC ranged from 0.35 (ESSR Chr6 26.5) to 0.76 (RMES 4-2) with an average value of 0.59, given in tables 7 and 8. The amplification of EST-SSR markers RMES 4-1 and RMES 11-2 that exhibited good polymorphism is presented in figure 2.

EST-based SSR cluster analysis

The similarity coefficient ranged from 0.13 to 0.79. All the 15 genotypes were grouped into two major clusters C1 and C2 with 13% similarity among them (figure 3). The major cluster C1 had two genotypes IR64 and Jaya with 79% similarity and C2 is divided into clusters A and B at 20% similarity.

Correlation between morphological trait and marker-based clustering

A dendrogram constructed based on mean values of 12 morphological and molecular markers showed that the same genotypes are grouped in different clusters. Based on morphological diversity analysis genotypes are grouped into four clusters. The maximum interclustered distance was observed between II and III, followed by I and II. The heterosis study showed the maximum level of heterosis (16.22%) expressed between the combination APMS6A × RPHR 1005 (table 9; figure 4) and these belong to groups I and II, respectively (figure 3). Based on molecular data, these genotypes are grouped in C2 major cluster with different subgroups and APMS 6A, EPLT109, BCW56, RPHR 1005 and KMR3R were very distinct from other genotypes.

Heterosis

Standard heterosis was estimated among the 40 hybrids for 12 characters and is given in table 10. Results showed that standard heterosis over KRH2 for DFF was in the range from −10.43 to 4.74%. Thirty-four hybrids registered significant negative standard heterosis with hybrid check and maximum by IR 68897A × RPHR1004 (−10.43%). For days to maturity, 25 hybrids registered significant negative standard heterosis with a range of −7.75 (IR68897A × RPHR1004) to −2.21% (PUSA5A × RPHR1096) and the plant height range was −0.36 (IR58025A × EPLT109) to 30.99% (APMS6A × RPHR1096).

The range of standard heterosis varied from −27.86 (PUSA5A × RPHR1004) to 7.22% (APMS6A × KMR3R) over KRH2. As many as five hybrids were significant in the positive direction for the number of productive tillers per plant. It varied from −68.79 (APMS6A × EPLT109) to 396.55% (PUSA5A × RPHR1004) for the number of unproductive tillers per plant. One hybrid IR 58025A × BCW56 (4.50%) showed positive standard heterosis over KRH2 positive direction for panicle length. It ranged from −30.00 (APMS6A × SG 22-289-3) to 15.21% (PUSA5A × EPLT109) and 14 hybrids were in the positive direction for panicle weight over KRH2. None of the hybrids exhibited significant positive standard heterosis over KRH2 for the number of filled grains per panicle.

Most of the hybrids exhibited negative heterosis for the character spikelet fertility per cent. For 1000-grain weight, five hybrids showed positive significant standard heterosis and the highest was 16.28% (IR68897A × SG-22-289-3). When compared with the standard check KRH2, the standard heterosis ranged from −38.32 (PUSA5A × RPHR1004) to 8.40% (APMS6A × RPHR1005), with five

Table 5. Clustering of 15 rice genotypes based on Mahalanobis distances.

	Cluster	Genotypes	No. of genotypes
1	I	APMS6A, RPHR1004, PMS10A, IR68897A	4
2	II	IR58025A, RPHR1005, SG-22-289-3, KMR3R, PHR619-2	5
3	III	PUSA5A, IR64, Jaya	3
4	IV	RPHR1096, EPLT109, BCW56	3

Table 6. Cluster means from 15 genotypes of rice for 10 significant characters.

Clusters	DFF	Plant height (cm)	Productive tillers	Panicle length (cm)	Panicle weight (cm)	Filled grains/panicle	Spikelet fertility (%)	1000 Seed weight (g)	Grain yield/plant (g)	Productivity/ day
1 Cluster	102.5	92.24	17.93	24.64	3.04	129.6	78.7	19.93	19.91	0.14
2 Cluster	105.2	100.4	16.58	23.83	3.49	145.2	76.2	19.9	20.44	0.15
3 Cluster	101.6	86.13	21.06	24.65	3.68	158.5	82.9	22.95	24.83	0.19
4 Cluster	109.5	104.8	15.98	24.25	3.99	140.4	73.0	21.16	19.66	0.14
Mean	104.63	96.25	17.72	24.29	3.51	142.8	77.6	20.77	21.02	0.15

hybrids being in the positive direction and maximum of 8.4% for grain yield per plant. And two hybrids exhibited significant positive standard heterosis, namely APMS6A × RPHR1005 and IR58025A × BCW56 11.36% each for per day productivity.

Prediction of heterosis by correlating the molecular divergence and standard heterosis for different yield and yield-contributing characters

The correlation values between standard heterosis over KRH2 and CMP values based on EST-SSR markers for 40 hybrids revealed positive and significant correlation among the yield and yield-related characters studied, namely grain yield per plant ($r = 0.58$**), productivity per day ($r = 0.54$**), productive tillers ($r = 0.34$*) and panicle weight ($r = 0.42$**) (table 11).

Discussion

Prediction of heterosis is being practiced in maize for the first time and partially successful since heterosis is a complex phenomenon. In this study, morphological and molecular (EST-SSR) marker-based diversity analyses are being used to predict the heterosis by correlating the coefficient of molecular polymorphism based on EST-SSR markers of hybrids with standard heterosis over hybrid check KRH2 along with the combining ability of parental lines and hybrids, high mean values of morphological traits, morphological and molecular diversity analysis.

Many workers have emphasized on divergent parents for a higher yield/level of heterosis (Smith *et al*. 1990) since heterosis is measured in terms of yield in rice. The diverse genotypes are selected based on several parameters, namely pedigree, morphological, physiological, biological and molecular data (markers, SNPs, NGS etc.) (Smith *et al*. 1990; Zhang *et al*. 2010).

Divergence is measured using Mahalanobis D^2 statistics which provides information about amount of genetic diversity present in genotypes and narrows down the number of genotypes considered for study. The success and usefulness of Mahalanobis's analysis in quantifying genetic divergence has been used by several workers, such as Hedge and Patil (2000), in rice.

In this study, a total of 12 morphological characters were used for diversity analysis of 15 rice genotypes and they were grouped into four clusters. The maximum intracluster distance was recorded in cluster IV (861.47) followed by cluster I (288.21). The cluster II recorded relatively less (198.11) and cluster III with three genotypes had 'no' intracluster distance. It was reported that genotypes within a cluster having a high degree of divergence would produce more desirable breeding material for achieving maximum genetic advance with regard to yield *per se*, provided there is an adequate complementation (Mundhe *et al*. 2006).

Table 7. Polymorphic and heterozygosity information of EST-derived SSR markers.

	EST-SSR marker	No. of alleles	Polymorphic alleles	PIC	Heterozygosity
1	RMES 6-1	6	6	0.70	0.74
2	RMES 6-2	4	4	0.59	0.65
3	RMES 7-1	4	4	0.64	0.69
4	RMES 7-2	3	3	0.54	0.60
5	RMES 8-1	5	5	0.69	0.74
6	RMES 8-2	1	1	0.00	0.00
7	RMES 9-1	4	4	0.64	0.68
8	RMES 9-2	4	4	0.61	0.67
9	ESSR Chr5-20.9	4	4	0.62	0.68
10	RMES 1-2	5	5	0.66	0.70
11	RMES 3-2	7	7	0.74	0.76
12	RMES 11-1	4	4	0.69	0.74
13	ESSR Chr 6 26.5	2	2	0.35	0.44
14	ESSR Chr 12 23.4	2	2	0.37	0.50
15	UGRM20 1-1	1	1	0.00	0.00
16	UGRM 785	4	4	0.50	0.56
17	RMES 10-1	4	4	0.62	0.68
18	RMES 2-2	4	4	0.55	0.59
19	RMES 12-2	3	3	0.48	0.55
20	RMES 3-1	4	4	0.64	0.68
21	RMES 4-1	3	3	0.45	0.50
22	RMES 1-1	3	3	0.39	0.43
23	RMES 11-2	5	5	0.65	0.69
24	RMES 10-2	4	4	0.64	0.70
25	RMES 4-2	6	6	0.76	0.79

Table 8. Allelic distribution of EST-derived SSR markers among 15 genotypes.

	EST-SSR marker	No. of alleles	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
1	RMES 8-2	1	15	0	0	0	0	0	0
2	UGRM20 1-1	1	15	0	0	0	0	0	0
3	ESSR Chr 6 26.5	2	10	5	0	0	0	0	0
4	ESSR Chr 12 23.4	2	7	8	0	0	0	0	0
5	RMES 7-2	3	8	4	3	0	0	0	0
6	RMES 12-2	3	2	9	4	0	0	0	0
7	RMES 4-1	3	10	1	4	0	0	0	0
8	RMES 1-1	3	2	2	11	0	0	0	0
9	RMES 6-2	4	1	5	7	2	0	0	0
10	RMES 7-1	4	5	2	2	6	0	0	0
11	RMES 9-1	4	7	3	3	2	0	0	0
12	RMES 9-2	4	1	3	7	4	0	0	0
13	ESSR Chr5 20.9	4	1	5	6	3	0	0	0
14	RMES 11-1	4	3	5	4	3	0	0	0
15	UGRM 785	4	9	1	1	4	0	0	0
16	RMES 10-1	4	2	4	2	7	0	0	0
17	RMES 2-2	4	2	2	9	2	0	0	0
18	RMES 3-1	4	2	3	7	3	0	0	0
19	RMES 10-2	4	5	5	4	1	0	0	0
20	RMES 8-1	5	1	5	2	5	2	0	0
21	RMES 1-2	5	7	3	2	2	1	0	0
22	RMES 11-2	5	2	6	3	2	2	0	0
23	RMES 6-1	6	1	4	2	1	6	1	0
24	RMES 4-2	6	3	2	2	5	2	1	0
25	RMES 3-2	7	1	3	6	2	1	1	1

Based on the values of intercluster distance it was found that the highest divergence occurred between clusters II and III (3202.41) followed by clusters II and I (1561.76), clusters II and IV (1366.75), clusters III and IV (1318.18) and clusters IV and I (985.74) indicating the wider genetic diversity between the genotypes of these groups.

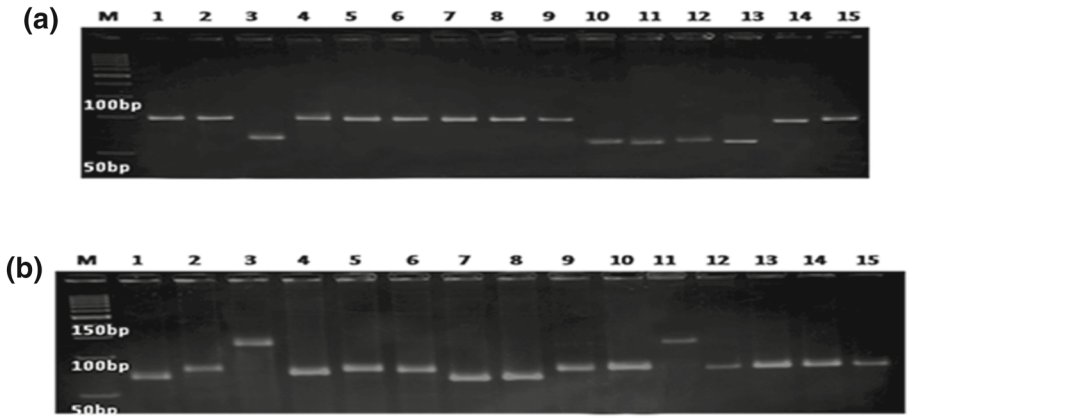


Figure 2. Amplification pattern of (a) RMES 4-1 and (b) RMES 11-2 markers.

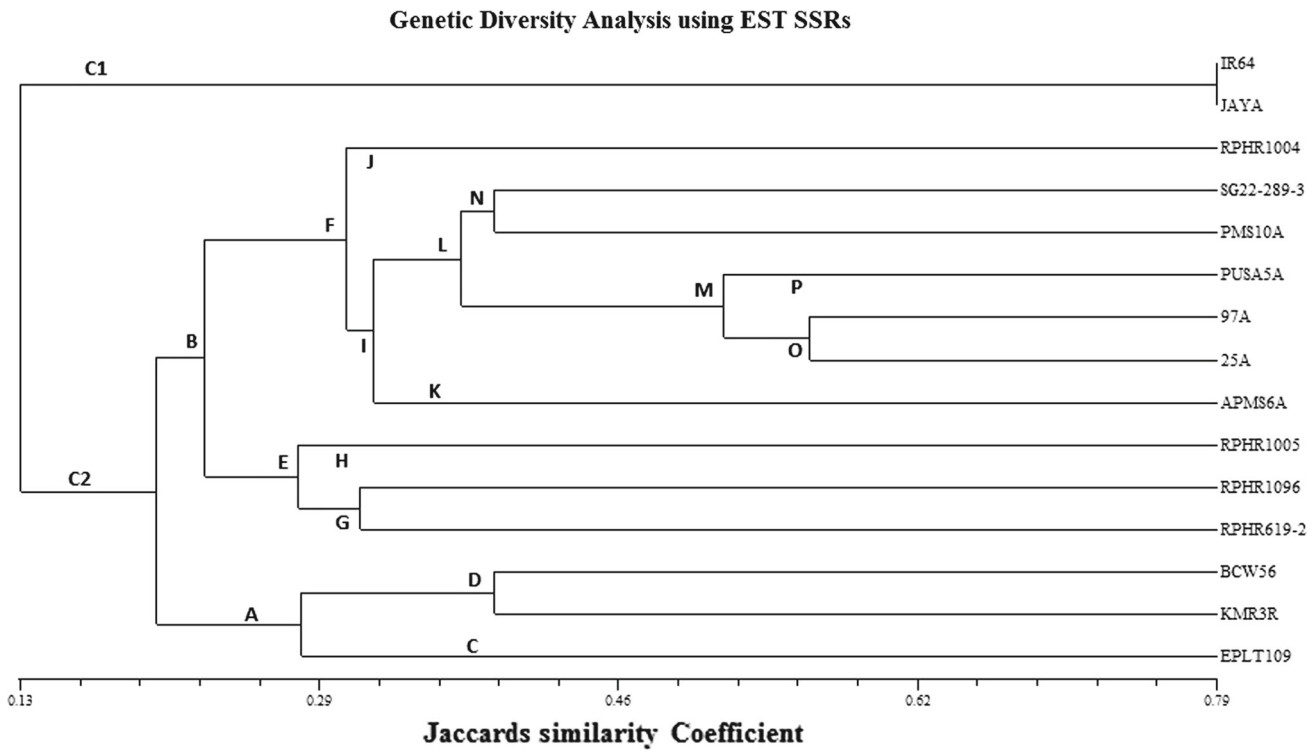


Figure 3. Dendrogram of the parental genotypes of the hybrids based on the EST-derived SSR markers.

Chand *et al.* (2005) and Chaturvedi and Maurya (2005) recommended that the parents should be selected from two clusters having a wider intercluster distance to realize much variability and a high-heterotic effect.

The maximum intercluster distance was observed between II and III, followed by I and II. But, the maximum level of heterosis (16.22%) was expressed between the combination APMS 6A × RPHR 1005 although these belong to groups I and II, respectively. This is mainly because of many factors including genetic factors and environment, their interactions, lesser number of traits studied, experimental errors, less diverse traits studied, error in selection of traits, complex traits under study, epistatic gene interactions, linkage problem etc. (Schut *et al.* 1997) which reduce the selection efficiency of parents based on morphological trait diversity analysis. These results are in conformity with the findings of Madhavilatha *et al.* (2005) and Mundhe *et al.* (2006).

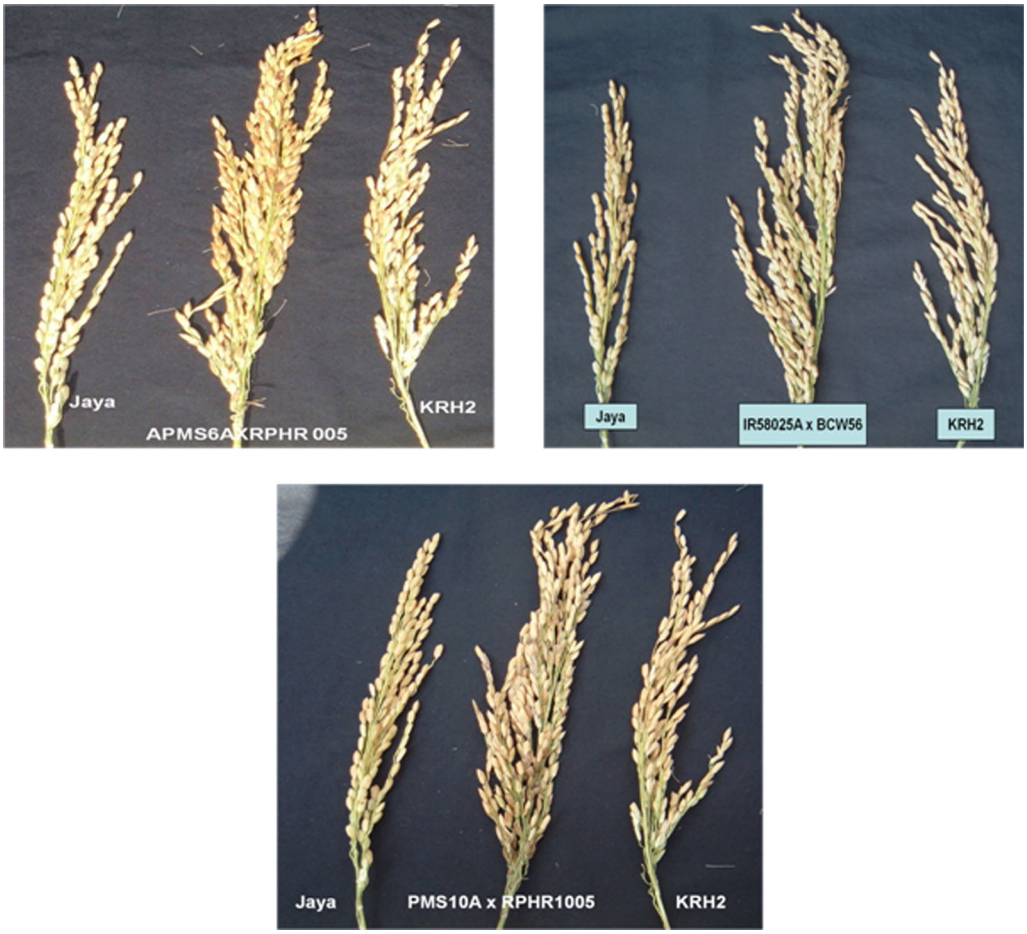


Figure 4. Top three high-yielding hybrids.

Table 9. Top three heterotic hybrids' overall performance for the trait grain yield per plant.

	Hybrid	Mean performance	Average heterosis (%)	Heterobeltiosis (%)	Standard heterosis (%) (over KRH2)	Standard heterosis (%) (over Jaya)	*Sca* effect
1	APMS6A × RPHR1005	32.25	58.67**	53.21**	**8.40**	**16.22****	3.3637
2	IR58025A × BCW56	31.70	68.39**	64.68**	**6.55**	**14.23****	4.8950
3	PMS10A × RPHR1005	31.55	45.73**	41.8**	**6.05**	**13.69***	2.4887

** And *significant at $P = 0.01$ and 0.05, respectively. Significant heterosis are in bold

Due to drawbacks in morphological data-based diversity analysis and advancement of molecular techniques, the efficiency of plant breeding has significantly increased (Tanksley *et al.* 1992). Among molecular markers, SSRs (which are also known as microsatellites) are more reliable, practical and highly efficient in detecting polymorphism (Stuber *et al.* 1992). But, the heterosis is measured mainly by yield in rice. Hence, the use of markers within genes (EST-SSRs) is more reliable for prediction of heterosis.

In this study, 25 EST-derived SSR primer pairs were used, of which 23 EST-SSR primers were found to be polymorphic and generated a total of 375 alleles and number of alleles per marker ranged 1–7 alleles per primer with an average of 3.84. A maximum of seven alleles were amplified by RMES 3-2 while, RMES 8-2 and UGRM 20 1-1 were found to be monomorphic. The primers showed an average PIC value of 0.59 which confirms the fact that the EST-SSR primers used in this study were highly informative in terms of heterosis prediction. Markers with the PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (DeWoody *et al.* 1995).

Table 10. Estimation of heterosis and their significance presented character wise.

		Standard heterosis											
		Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya
	Crosses	DFF		Days to maturity		Plant height (cm)		No. of productive tillers		No. of un productive tillers		Panicle length (cm)	
1	APMS6-A*RPHR1004	−4.74**	−1.95	−3.32**	−1.50	2.41	9.79**	−14.76**	−27.38**	−27.59	31.25	−8.34*	0.53
2	APMS6-A*RPHR1005	−4.27**	−1.46	−2.58**	−0.75*	9.43**	17.31**	−6.09*	−20.00**	−25.86	34.38	−1.41	8.14
3	APMS6-A*RPHR1096	−2.84**	0.00	−0.74	1.13**	30.99**	40.42**	−18.67**	−30.71**	35.69	145.94	−2.68	6.74
4	APMS6-A*BCW56	−6.16**	−3.41**	−2.58**	−0.75*	23.96**	32.89**	−6.25*	−20.13**	−40.52	7.81	−6.20	2.89
5	APMS6-A*KMR-3R	−5.21**	−2.44*	−2.58**	−0.75*	15.80**	24.14**	7.22*	−8.65**	−28.79	29.06	−2.86	6.54
6	APMS6-A*EPLT109	−2.84**	0.00	0.00	1.88**	6.41**	14.07**	−11.29**	−24.42**	−68.79	−43.44	−0.59	9.03*
7	APMS6-A*SG-22-289-3	−5.21**	−2.44*	−3.32**	−1.50	10.52**	18.48**	−15.12**	−27.69**	228.62**	495.63**	−7.50	1.46
8	APMS6-A*RPHR619-2	−1.90	0.98	−0.74	1.13**	1.93	9.27**	−11.74**	−24.81**	−5.69	70.94	−5.93	3.18
9	PMS10-A*RPHR1004	−0.95	1.95	0.00	1.88**	0.69	7.95**	−19.41**	−31.35**	−25.34	35.31	−3.78	5.53
10	PMS10-A*RPHR1005	0.00	2.93**	0.00	1.88**	4.60**	12.13**	0.07	−14.75**	−53.97	−16.56	−0.74	8.87*
11	PMS10-A*RPHR1096	0.00	2.93**	0.74	2.63**	5.47**	13.07**	−9.71**	−23.08**	−39.14	10.31	−5.51	3.64
12	PMS10-A*RPHRBCW56	−2.37*	0.49	−1.48	0.38**	15.89**	24.23**	−10.84**	−24.04**	−40.86	7.19	−6.76	2.27
13	PMS10-A*KMR-3R	4.74**	7.80**	4.06**	6.02**	10.50**	18.46**	−20.99**	−32.69**	−13.62	56.56	−5.08	4.11
14	PMS10-A*EPLT109	−0.47	2.44*	−0.37	1.50**	14.22**	22.45**	−1.35	−15.96**	11.55	102.19	−1.42	8.12
15	PMS10-A*SG-22-289-3	−2.37*	0.49	−1.48	0.38**	21.93**	30.71**	−13.48**	−26.29**	−45.86	−1.88	−6.43	2.63
16	PMS10-A*RPHR619-2	−2.37*	0.49	−1.48	0.38**	15.94**	24.29**	−19.19**	−31.15**	112.76	285.63**	−2.70	6.72
17	PUSA5-A*RPHR1004	−3.79**	−0.98	−2.58**	−0.75*	1.65	8.97**	−27.86**	−38.54**	396.55**	800.00**	−9.51*	−0.75
18	PUSA5-A*RPHR1005	−5.21**	−2.44*	−3.69**	−1.88	5.21**	12.79**	−20.18**	−32.00**	−63.45	−33.75	−4.00	5.30
19	PUSA5-A*RPHR1096	−3.79**	−0.98	−2.21*	−0.38**	18.13**	26.63**	−24.60**	−35.77**	47.07	166.56	−4.95	4.25
20	PUSA5-A*BCW56	−4.74**	−1.95	−3.32**	−1.50	20.94**	29.65**	−9.71**	−23.08**	−31.03	25.00	−2.90	6.50
21	PUSA5-A*KMR-3R	−5.69**	−2.93**	−3.32**	−1.50	7.81**	15.58**	−17.88**	−30.04**	−66.55	−39.38	−3.12	6.26
22	PUSA5-A*EPLT109	−2.84**	0.00	−1.85	0.00**	5.89**	13.51**	−5.12	−19.17**	−47.93	−5.63	−1.96	7.53
23	PUSA5-A*SG-22-289-3	−6.16**	−3.41**	−4.80**	−3.01	2.60	9.99**	−15.67**	−28.15**	−19.31	46.25	−5.80	3.32
24	PUSA5-A*RPHR619-2	−4.27**	−1.46	−3.32**	−1.50	5.73**	13.34**	−19.41**	−31.35**	43.10	159.38	−4.04	5.26
25	IR68897-A*RPHR1004	−10.43**	−7.80**	−7.75**	−6.02**	2.95	10.36**	−10.16**	−23.46**	6.90	93.75	−6.18	2.91
26	IR68897-A*RPHR1005	−9.48**	−6.83**	−7.38**	−5.64**	5.68**	13.29**	0.23	−14.62**	−3.28	75.31	−6.14	2.94
27	IR68897-A*RPHR1096	−7.11**	−4.39**	−4.80**	−3.01	11.09**	19.10**	−7.90*	−21.54**	−8.62	65.63	−4.07	5.22
28	IR68897-A*BCW56	−6.16**	−3.41**	−4.06**	−2.26	25.83**	34.90**	−19.64**	−31.54**	−18.28	48.13	−3.21	6.17
29	IR68897-A*KMR-3R	−6.16**	−3.41**	−4.80**	−3.01	5.57**	13.18**	−19.05**	−31.04**	−26.21	33.75	−2.54	6.90
30	IR68897-A*EPLT109	−6.64**	−3.90**	−4.80**	−3.01	1.56	8.88**	−24.83**	−35.96**	−25.86	34.38	−5.35	3.81
31	IR68897-A*SG-22-289-3	−8.53**	−5.85**	−5.54**	−3.76	2.66	10.05**	−25.51**	−36.54**	39.66	153.13	−5.48	3.68
32	IR68897-A*RPHR619-2	−8.06**	−5.37**	−6.27**	−4.51	10.73**	18.70**	−15.12**	−27.69**	−25.86	34.38	−7.69	1.25
33	IR58025-A*RPHR1004	−3.79**	−0.98	−2.95**	−1.13	2.52	9.90**	−23.70**	−35.00**	−16.38	51.56	−3.91	5.40
34	IR58025-A*RPHR1005	−1.90	0.98	−0.74	1.13**	4.44**	11.97**	−2.71	−17.12**	84.48	234.38*	−2.67	6.76

Table 10 (*contd*)

Crosses	Standard heterosis											
	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya
	DFF		Days to maturity		Plant height (cm)		No. of productive tillers		No. of un productive tillers		Panicle length (cm)	
35 IR58025-A*RPHR1096	−2.84**	0.00	−1.48	0.38**	1.56	8.88**	−22.89**	−34.31**	0.00	81.25	−8.02	0.89
36 IR58025-A*BCW56	−4.74**	−1.95	−3.32**	−1.50	8.44**	16.25**	−5.96	−19.88**	−25.17	35.63	4.50	14.62**
37 IR58025-A*KMR-3R	−2.84**	0.00	−2.58**	−0.75*	5.78**	13.40**	0.45	−14.42**	−5.17	71.88	−2.54	6.90
38 IR58025-A*EPLT109	3.32**	6.34**	2.58**	4.51**	−0.36	6.81**	−9.71**	−23.08**	−37.93	12.50	−4.95	4.25
39 IR58025-A*SG-22-289-3	−6.16**	−3.41**	−4.80**	−3.01	6.93**	14.63**	−19.05**	−31.04**	9.31	98.13	−4.70	4.53
40 IR58025-A*RPHR619-2	−3.79**	−0.98	−2.58**	−0.75*	4.32**	11.84**	−10.84**	−24.04**	52.07	175.63	−3.86	5.45

	Standard heterosis											
	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya
	Panicle weight (g)		No. of filled grains per panicle		Spikelet fertility percentage		1000 Seed weight (g)		Productivity per day (g)		Grain yield per single plant (g)	
1 APMS6-A*RPHR1004	−15.11	−16.26*	−13.33	−5.52	−13.30	−11.94	−11.63*	−25.78**	−13.64*	−9.52	−15.97**	−9.91
2 APMS6-A*RPHR1005	−7.13	−8.39	−4.82	3.77	−10.26	−8.86	−13.95**	−27.73**	11.36*	16.67**	**8.40**	**16.22****
3 APMS6-A*RPHR1096	0.53	−0.84	−14.33	−6.60	−5.29	−3.81	0.00	−16.02**	−13.64*	−9.52	−14.29**	−8.11
4 APMS6-A*BCW56	−4.04	−5.35	−19.98	−12.76	−15.34	−14.01	6.98	−10.16**	−20.45**	−16.67**	−22.69**	−17.12**
5 APMS6-A*KMR-3R	5.85	4.41	−5.28	3.26	0.06	1.63	−4.65	−19.92**	2.27	7.14	**0.84**	**8.11**
6 APMS6-A*EPLT109	8.83	7.35	−5.55	2.97	−0.66	0.89	−2.33	−17.97**	−2.27	2.38	−2.52	4.50
7 APMS6-A*SG-22-289-3	−30.00**	−30.95**	−28.44*	−21.99	−20.98*	−19.74*	−9.30*	−23.83**	−25.00**	−21.43**	−27.39**	−22.16**
8 APMS6-A*RPHR619-2	−0.74	−2.10	−10.36	−2.28	−12.77	−11.40	4.65	−12.11**	−11.36*‘	−7.14	−11.26*	−4.86
9 PMS10-A*RPHR1004	−6.91	−8.18	−17.59	−10.16	−8.72	−7.29	−9.30*	−23.83**	−13.64*	−9.52	−14.62**	−8.47
10 PMS10-A*RPHR1005	1.06	−0.31	−0.56	8.41	−2.18	−0.65	−13.95**	−27.73**	6.82	11.90*	**6.05**	**13.69***
11 PMS10-A*RPHR1096	−2.98	−4.30	−15.01	−7.35	−8.93	−7.51	0.00	−16.02**	−13.64*	−9.52	−11.93*	−5.59
12 PMS10-A*RPHRBCW56	6.70	5.25	−3.84	4.83	−11.03	−9.64	13.95**	−4.30	−9.09	−4.76	−8.74	−2.16
13 PMS10-A*KMR-3R	−11.91	−13.12	−12.68	−4.80	−18.79*	−17.52*	0.00	−16.02**	−18.18**	−14.29*	−15.63**	−9.55

Table 10 (*contd*)

		Standard heterosis											
		Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya	Over KRH2	Over Jaya
		Panicle weight (g)		No. of filled grains per panicle		Spikelet fertility percentage		1000 Seed weight (g)		Productivity per day (g)		Grain yield per single plant (g)	
14	PMS10-A*EPLT109	14.89	13.33	−5.23	3.32	−2.83	−1.31	9.30*	−8.20*	0.00	4.76	−1.68	5.41
15	PMS10-A*SG-22-289-3	−16.38	−17.52*	−21.87	−14.83	−9.84	−8.43	−11.63*	−25.78**	−18.18**	−14.29*	−19.66**	−13.87*
16	PMS10-A*RPHR619-2	−16.06	−17.21*	−28.15*	−21.67	−18.54*	−17.27*	0.00	−16.02**	−11.36*	−7.14	−13.95**	−7.75
17	PUSA5-A*RPHR1004	−23.94**	−24.97**	−24.18	−17.35	−15.40	−14.08	−9.30*	−23.83**	−36.36**	−33.33**	−38.32**	−33.87**
18	PUSA5-A*RPHR1005	−14.79	−15.95	−23.87	−17.00	−17.02*	−15.72	−6.98	−21.88**	−11.36*	−7.14	−13.28**	−7.03
19	PUSA5-A*RPHR1096	−7.55	−8.81	−17.76	−10.34	−11.17	−9.78	−2.33	−17.97**	−25.00**	−21.43**	−25.21**	−19.82**
20	PUSA5-A*BCW56	−0.64	−1.99	−14.99	−7.32	−15.36	−14.04	9.30*	−8.20*	−11.36*	−7.14	−13.45**	−7.21
21	PUSA5-A*KMR-3R	1.38	0.00	−5.50	3.02	−7.44	−5.99	−4.65	−19.92**	−11.36*	−7.14	−14.29**	−8.11
22	PUSA5-A*EPLT109	15.21	13.64	−6.88	1.52	−6.68	−5.22	6.98	−10.16**	2.27	7.14	**0.00**	**7.21**
23	PUSA5-A*SG-22-289-3	−9.15	−10.39	−19.17	−11.88	−23.14**	−21.94*	9.30*	−8.20*	−25.00**	−21.43**	−28.57**	−23.42**
24	PUSA5-A*RPHR619-2	−14.15	−15.32	−20.90	−13.77	−14.68	−13.35	−4.65	−19.92**	−18.18**	−14.29*	−22.18**	−16.58**
25	IR68897-A*RPHR1004	−5.32	−6.61	−3.38	5.33	−7.65	−6.20	−2.33	−17.97**	−2.27	2.38	−9.24	−2.70
26	IR68897-A*RPHR1005	−24.68**	−25.71**	−36.55**	−30.82*	−28.55**	−27.44**	−2.33	−17.97**	−18.18**	−14.29*	−25.21**	−19.82**
27	IR68897-A*RPHR1096	−6.81	−8.08	4.23	13.63	−5.18	−3.69	−13.95**	−27.73**	−6.82	−2.38	−10.42*	−3.96
28	IR68897-A*BCW56	−9.79	−11.02	−32.36*	−26.26	−21.76*	−20.53*	6.98	−10.16**	−20.45**	−16.67**	−24.03**	−18.56**
29	IR68897-A*KMR-3R	−11.49	−12.70	−16.90	−9.41	−11.23	−9.84	−16.28**	−29.69**	−6.82	−2.38	−10.42*	−3.96
30	IR68897-A*EPLT109	0.21	−1.15	−10.92	−2.89	−10.33	−8.93	0.00	−16.02**	−9.09	−4.76	−13.45**	−7.21
31	IR68897-A*SG-22-289-3	−0.85	−2.20	−21.58	−14.51	−12.97	−11.60	16.28**	−2.34	−29.55**	−26.19**	−32.77**	−27.93**
32	IR68897-A*RPHR619-2	10.21	8.71	−10.46	−2.39	−4.55	−3.06	2.33	−14.06**	−4.55	0.00	−10.59*	−4.14
33	IR58025-A*RPHR1004	−12.45	−13.64	−22.29	−15.28	−9.76	−8.34	−16.28**	−29.69**	−13.64*	−9.52	−15.13**	−9.01
34	IR58025-A*RPHR1005	5.11	3.67	−6.86	1.54	−2.34	−0.81	0.00	−16.02**	−4.55	0.00	−5.55	1.26
35	IR58025-A*RPHR1096	−16.17	−17.31*	−19.12	−11.83	−17.15*	−15.85	−9.30*	−23.83**	−15.91**	−11.90*	−16.81**	−10.81*
36	IR58025-A*BCW56	8.72	7.24	−11.24	−3.24	−13.36	−12.00	2.33	−14.06**	11.36*	16.67**	**6.55**	**14.23****
37	IR58025-A*KMR-3R	−7.77	−9.02	−16.45	−8.91	−15.37	−14.04	−4.65	−19.92**	−4.55	0.00	−5.88	0.90
38	IR58025-A*EPLT109	3.19	1.78	−18.35	−10.98	−10.54	−9.14	4.65	−12.11**	−11.36*	−7.14	−9.58	−3.06
39	IR58025-A*SG-22-289-3	−15.53	−16.68*	−15.18	−7.53	−20.81*	−19.57*	−4.65	−19.92**	−22.73**	−19.05**	−25.71**	−20.36**
40	IR58025-A*RPHR619-2	2.23	0.84	−9.54	−1.38	−8.86	−7.43	6.98	−10.16**	−13.64*	−9.52	−16.30**	−10.27

** And *significant at $P = 0.01$ and 0.05, respectively. Significant heterosis are in bold

Table 11. CMP for EST-SSRs for the F_1 rice hybrids.

	Crosses	Total no. of markers	Polymorphic markers	Monomorphic markers	CMP
1	APMS6A × RPHR1004	25	15	10	0.60
2	APMS6A × RPHR1005	25	20	5	0.80
3	APMS6A × RPHR1096	25	18	7	0.72
4	APMS6A × BCW56	25	17	8	0.68
5	APMS6A × KMR3R	25	16	9	0.64
6	APMS6A × EPLT109	25	13	12	0.52
7	APMS6A × SG-22-289-3	25	12	13	0.48
8	APMS6A × RPHR619-2	25	19	6	0.76
9	PMS10A × RPHR1004	25	15	10	0.60
10	PMS10A × RPHR1005	25	17	8	0.68
11	PMS10A × RPHR1096	25	14	11	0.56
12	PMS10A × BCW56	25	16	9	0.64
13	PMS10A × KMR3R	25	18	7	0.72
14	PMS10A × EPLT109	25	15	10	0.60
15	PMS10A × SG-22-289-3	25	11	14	0.44
16	PMS10A × RPHR619-2	25	16	9	0.64
17	PUSA5A × RPHR1004	25	10	15	0.40
18	PUSA5A × RPHR1005	25	14	11	0.56
19	PUSA5A × RPHR1096	25	13	12	0.52
20	PUSA5A × BCW56	25	16	9	0.64
21	PUSA5A × KMR3R	25	18	7	0.72
22	PUSA5A × EPLT109	25	20	5	0.80
23	PUSA5A × SG-22-289-3	25	13	12	0.52
24	PUSA5A × RPHR619-2	25	15	10	0.60
25	IR68897A × RPHR1004	25	13	12	0.52
26	IR68897A × RPHR1005	25	15	10	0.60
27	IR68897A × RPHR1096	25	15	10	0.60
28	IR68897A × BCW56	25	16	9	0.64
29	IR68897-A*KMR-3R	25	16	9	0.64
30	IR68897-A*EPLT109	25	17	8	0.68
31	IR68897-A*SG-22-289-3	25	13	12	0.52
32	IR68897-A*RPHR619-2	25	13	12	0.52
33	IR58025-A*RPHR1004	25	13	12	0.52
34	IR58025-A*RPHR1005	25	14	11	0.56
35	IR58025-A*RPHR1096	25	15	10	0.60
36	IR58025-A*BCW56	25	17	8	0.68
37	IR58025-A*KMR-3R	25	17	8	0.68
38	IR58025-A*EPLT109	25	18	7	0.72
39	IR58025-A*SG-22-289-3	25	11	14	0.44
40	IR58025-A*RPHR619-2	25	15	10	0.60

The dendrogram was constructed based on similarity matrices using data from all the 23 polymorphic EST-SSR markers and 15 genotypes were grouped into two major clusters. The range of similarity coefficient between the genotypes is 0.13–0.79 with an average of 0.46. However, a maximum degree of similarity of 52% was obtained between lines IR58025A and IR68897A and 28% among restorer lines RPHR 1005, EPLT109, KMR3R and BCW56. The degree of similarity between male sterile and restorer line ranged by 0.20–0.58. The dendrogram indicated restorer lines, namely RPHR 1005, EPLT109, KMR3R and BCW56 and CMS line APMS 6A were very distinct among all the genotypes and hybrid generated from these showed high yields. This indicates that the molecular-based diversity analysis is more reliable compared to morphological-based diversity analysis. EST-SSRs are not influenced by the environment, transcribed regions of DNA, higher rate of transferability across species and more conserved regions in genes expressing yield-related traits.

But, heterosis study showed the maximum level of heterosis in APMS6A × RPHR 1005, IR58025A × BCW56 and PMS10A × RPHR1005 and significant positive standard heterosis of 8.4, 6.55 and 6.05, respectively, over the KRH2 and 16.22, 14.23 and 13.69, respectively, over Jaya although IR58025A and PMS10A are in different groups (figure 4). This is because heterosis is a complex phenomenon and other reasons may include a narrow range of genetic diversity with respect to the genomic regions analysed with EST-SSR markers among the selected parents, using less number of markers, poor association between the marker and gene/QTLs with

heterosis, interaction among genes/QTLs, error in selection of traits and experimental materials, experimental error, epigenetic effects, lack of statistical model, environment effects, functional genomics etc. (Melchinger *et al*. 1990; Zha 2008; Zhang *et al*. 2010).

Prediction of heterosis by correlating the molecular divergence and standard heterosis for different yield and yield-contributing characters

Correlation study between CMP based on EST-SSR markers and standard heterosis of experimental hybrid was used to predict the grain yield heterosis. Zhang *et al*. (1994) proposed that specific heterozygosity with respect to positive markers (with significant effects on the target trait) may show higher positive correlation with heterosis for grain yield. Joshi *et al*. (2004) study also confirmed the utility of specific SSR markers, which displayed significant correlation with hybrid performance. The result obtained from the present study has also validated the concept of key DNA markers for prediction of heterosis proposed by Cho *et al*. (2004) and SSR loci proposed by Zhang *et al*. (2008). Zhang *et al*. (2010) predicted heterosis based on the yield-related functional genes.

In this study, CMP value ranges from 0.40 to 0.80 among the 40 parental combinations, a higher CMP value (0.80) is obtained for APMS6A × RPHR1005 and PUSA5A × EPLT109, while a low CMP value (0.40) is observed for PUSA5A × RPHR1004. The standard heterosis of all the 40 F_1 hybrids ranged from −38.32 (PUSA5A × RPHR1004) to 8.4% (APMS6A × RPHR1005) for grain yield per plant. The correlation study between CMP and standard heterosis for grain yield and other yield-contributing traits indicated positive values for parental combinations and significant grain yield per plant (r = 0.58**), productivity per day (r = 0.54**), productive tillers (r = 0.34*) and panicle weight (r = 0.42**).

This study revealed that high correlation between significant CMP value and standard heterosis can be utilized for prediction of heterosis for yield and yield-contributing traits in rice.

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