

Original Research Article

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Prediction of Heterosis Using Hypervariable Microsatellite Markers in Tropical *japonica* × *indica* Rice Hybrids

Sandeep Kumar Soni^{1*}, V.K. Yadav¹, V.P. Bhadana², M.C. Yadav³ and R.M. Sundaram⁴

¹Department of Genetics and Plant Breeding, C. S. Azad University of Agriculture and Technology, Kanpur-208 002, U.P., India

²ICAR-Indian Institute of Agricultural Biotechnology, Ranchi – 834010, Jharkhand, India

³Division of Genomic Resources, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-11002, India

⁴Indian Institute of Rice Research, Rajendranagar, Hyderabad- 500 030, Telangana, India

*Corresponding author

ABSTRACT

Keywords

Informative Microsatellite markers, Standard heterosis, Coefficient of marker polymorphism, Yield efficiency, Heterosis predicibility.

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Hybrid breeding programs involves large number of combinations made and evaluated, only a few are selected. Therefore, it is very important to explore alternative approaches to enhance the selection efficiency and to predict hybrid performance. In the present study, a set of 30 hybrids derived from crosses between 10 *tropical japonica* lines and 3 *indica* were analyzed for heterosis, per-day productivity, yield efficiency and an association index were established between yield and molecular marker amplification pattern. The heterosis value varied from -80.73% to 14.95% over the selected standard check. Parental lines of all the hybrids were characterized using a set of 40 uniformly distributed hypervariable microsatellite markers. A total of 131 allelic fragments with an average of 3.28 alleles per locus amplified, indicated presence of polymorphism among the rice lines. Similarity coefficients ranged from 0.58 to 0.83. Coefficient of marker polymorphism (CMP) for each hybrid was calculated based on extent of polymorphism among the parental lines of the hybrid with respect to markers analyzed. Association of CMP with the standard heterosis for grain yield, showed positive correlation ($r = 0.227$), indicating the strong utility of the selected microsatellite markers with $PIC \geq 0.5$ for prediction of heterosis.

Introduction

A large number of combinations annually made and evaluated in hybrid breeding programs, only a few hybrids are selected. This means extremely intensive labor is required, time is consumed and the selection efficiency is low. Thus, it is very important to explore alternative approaches to enhance the selection efficiency and to predict hybrid performance, such as through combining ability analysis and genetic distance on the

basis of genomic variation of the parental lines. Hybrid rice technology is one of the feasible options to meet the future food demand of 852 million tons in 2035, we need to enhance rice production to current production level of 676 million tons to fillip the dietary requirements of ever growing populations (Khush, 2013). The potential of this technology has been successfully employed in China. Conservative evidence

indicated that for increasing hybrid breeding efficiency, diverse data sets, such as morphology (Bar-Hen *et al.*, 1995), isozymes (Hamrick and Godt 1997), storage protein profiles (Smith *et al.*, 1987), pedigree records (de Leon and Carpena 1995) and DNA markers (Zhang *et al.*, 1994, Cho *et al.*, 2004) have been employed for assessing the parental genetic diversity, which were then correlated with grain yield heterosis. Among the different classes of molecular markers deployed in rice, simple sequence repeats (SSRs) are the most favored for a variety of applications in genetics and breeding because of their multi-allelic nature, high reproducibility, co-dominant inheritance, abundance and extensive genome coverage (McCouch *et al.*, 2002). Recently, the focus in crop genetics is being made towards a special class of SSRs, markers (Varshney *et al.*, 2005).

Materials and Methods

Plant materials

Ten *tropical japonica* lines (TJ – 5769, TJ – 64897, TJ – 25966, TJ – 4122, TJ – 11010, TJ – 16081, TJ – 16073, TJ – 25892, TJ – 10365 and TJ – 39050) and three testers namely NDR-359, CSR-36 and PB-1 were used to develop 30 F₁'s following the Line × Tester fashion.

Calculation of heterosis

The hybrids along with their parents and a standard check, NDR359 were grown at C.S. Azad University of Agriculture and Technology, Kanpur (UP) in 5 rows of 3 meter length with intra and inter spacing of 20 × 30 cm for grain yield and other yield contributing observations were recorded: days to 50% flowering, plant height, number of productive tillers per plant spikelet fertility and grain yield per plant in a Randomized

Complete Block Design (RCBD). Standard heterosis of the hybrids over the check variety NDR 359 was computed for grain yield and per-day productivity. Only the hybrids with spikelet fertility < 77% were considered for the analysis.

Molecular marker assay and data analysis

Total genomic DNA was isolated from the growing pots at 25 days young leaves of the parental lines following CTAB extraction method (Murray and Thompson 1980) and some modification with Williams *et al.*, (1990). A set of 40 hypervariable SSR markers (listed in table 1) spread uniformly across the rice genome were selected analyzing the amplification pattern among the parental lines at Indian Rice Research Institute (IRRI) Rajindranagar, Hyderabad, Telangana during *Rabi* 2012-13.

PCR amplification was carried out using the selected markers using the protocol described by Jaikishen *et al.*, (2010) and the amplicons were resolved on a 4 % Seakem® LE agarose gel (Lonza USA), stained with ethidium bromide. Only the clear and unambiguous distinguishable bands were used in genetic analysis. Molecular weight/Band size was estimated by comparing with 100bp ladder (Invitrogen, USA) using Totallab, (Nonlinear Dynamics Ltd, USA). Polymorphism information content (PIC) values at each locus were calculated according to Botstein *et al.*, (1980).

The coefficient of marker polymorphism for each hybrid was calculated as described in Jaikishen *et al.*, (2010). Values of correlation between CMP and standard heterosis for grain yield (of the selected hybrids possessing spikelet fertility of < 77%) was calculated for both EST and genomic SSRs using the correlation function from Microsoft excel package.

Results and Discussion

Heterosis of the experimental hybrids

The highest grain yield per plant in line \times tester cross was recorded for the tester NDR-359 (30.43g) followed by the most promising lines TJ-11010 (24.91g) and TJ-16081 (22.39g) have also very good spikelet fertility percent of 85.31 and 83.16, early flowering and short plant stature respectively (Table 2). While parental line TJ-39050 exhibited the least grain yield of (5.59g). Per day productivity percent of 30 test hybrids ranged from -18.62 (TJ – 64897 \times NDR-359) to 18.44 (TJ – 10365 \times NDR-359). The heterosis for grain yield per plant of hybrids over better parent ranged from -65.69 (TJ – 64897 \times NDR-359) to 40.39 (TJ – 11010 \times PB-1), mid parent from -47.66 (TJ – 64897 \times NDR-359) to 47.03 (TJ – 16081 \times NDR-359) and standard heterosis ranged from -80.73 (TJ – 39050 \times PB-1) to 14.95 (TJ – 11010 \times PB-1). The hybrid TJ – 11010 \times PB-1 showed a maximum heterobeltiosis of 40.39% followed by TJ – 11010 \times CSR-36 (33.76%) and TJ – 25892 \times CSR-36 (32.03%). The least positive heterobeltiosis was observed for the hybrid TJ – 5769 \times CSR-36 (0.83%). A higher magnitude of heterosis over mid parent was calculated for the hybrid TJ – 16081 \times NDR-359 (47.03%), followed by TJ – 25892 \times PB-1 (37.72%) and TJ – 11010 \times PB-1 (35.32%). The three test hybrid TJ-11010 \times PB-1 (14.95) followed by TJ-11010 \times NDR-359 (13.41%) and TJ-11010 \times CSR-36 (9.52%) showed the highest grain yield heterosis over standard check variety NDR-359 respectively. The least yield heterosis in negative direction over the standard check variety NDR-359 was calculated for the hybrid TJ – 39050 \times PB-1 having 80.73% and also have low panicle bearing tillers per plant of 5.45. The hybrid TJ – 64897 \times NDR-359 showed very early days to flowering having 75 days followed by the hybrid TJ – 25966 \times CSR-36 with 83 days and TJ – 25892 \times CSR-36 for 87 days to

flowering. The shortest stature of plant was exhibited by the hybrid TJ – 4122 \times CSR-36 having 94 cm and TJ – 4122 \times NDR-359 with 99 cm of plant height. The hybrid TJ – 11010 \times PB-1 showed highest spikelet fertility of 89.77% followed by TJ – 11010 \times NDR-359 (89.90%) and TJ – 25892 \times PB-1 (87.73%) of while least of 77.27 % spikelet fertility was recorded for the hybrid TJ – 64897 \times NDR-359.

Molecular marker amplification pattern among the parental lines analyzed

A total of forty three used primer pairs, 40 amplified a total of 131 fragments (alleles) with an average of 3.28 alleles per primer pair (Table 1). All the SSR markers used in the present study were polymorphic among the 13 parents analyzed (10 lines and 3 testers). The total numbers of bands were observed for each primer and polymorphism information content was calculated separately for each primer. Majority of the primer amplified three alleles at a locus. The primer pairs, i.e. HRM 16592 and HRM 25796 amplified a maximum of 5 bands, while primer pairs HRM 18770, HRM 20948, HRM 25355, HRM 27323, HRM 26213, HRM 27322 and HRM 27183 amplified 4 bands at a locus. The Polymorphic information content (PIC) values for the SSR loci ranged from 0.231 to 0.755 with an average of 0.542, which revealed that significant variation was present among the studied parental lines (Table 2).

The primer pair HRM 25796 showed high PIC value of 0.755; followed by HRM 26213 having 0.684 while the least PIC value of 0.231 was calculated by the primer HRM 17201. The similarity coefficients among the genotypes ranged from 0.58 to 0.83 exhibited presence of significant variations among the studied parental lines (Fig. 2). The UPGMA cluster analysis created two main clusters in which TJ-11010 was diverse to all the studied lines and stand in separate cluster also.

Table.1 SSR markers analyzed in the study, their amplification pattern and PIC values

S.N.	Primer	Chromosome No.	Primer sequence (5'–3')	Primer sequence (3'–5')	Total number of fragments amplified	PIC*	Fragment size range (bp)
1.	HRM 10615	1	accatggtatagtcctactaactcc	tagatgtatgagtcggaatggagtcg	3	0.568	150-250
2.	HRM 11111	1	gaagctactccaagttcagtcattgc	cgcccttagctgtgtattgtaatcg	3	0.459	350-400
3.	HRM 10936	1	cagacgcagtggtgtatgaagttcc	ggaccgattaaagtgaacgttgatgg	3	0.571	200-500
4.	HRM 13154	2	gcgggacattcatgtacacattgc	ctaggtctgtacacgcactgaagg	3	0.391	300-500
5.	HRM 13659	2	ggcttatcagtcatacaacagacg	tggttgggtatagcacggtaagc	3	0.631	100-400
6.	HRM 12469	2	gaacatgtcttgggtgtgatacagg	cttatcagctagcaccacaagttgg	3	0.604	150-300
7.	HRM 12349	2	ggacatggtgtctacttctctctcc	gggtatatatgcgtacaaggatgg	3	0.551	200-300
8.	HRM 15337	3	ttctatccctacagctctgacagc	cgttcaaggagcttgtgtgatcc	3	0.551	200-600
9.	HRM 15630	3	cgagccatcatacctccgtctagc	tgattcctctatatgcagccttgg	3	0.391	300-380
10.	HRM 16006	3	acgcttcacattgtaacacacagg	tctttgaaggctattcctggaacc	3	0.551	250-400
11.	HRM 17201	4	gcttaattctgacagaccagtcg	tggcctcttftttcatactcc	3	0.231	200-250
12.	HRM 17600	4	tcgccatctcaatccaactagc	ctgttctcgtttacaccttctgc	3	0.418	100-300
13.	HRM 16343	4	cgctcatctgaattgtgcttacc	tatgcacagccgggtacataatcc	3	0.551	200-500
14.	HRM 16592	4	cggtaaatactctggaacttcg	ggtttggttcactactcgtttgc	5	0.498	200-350
15.	HRM 18857	5	gggtacgtattagcaggtttcg	gatcgttctccttcaatgagg	3	0.587	100-300
16.	HRM 18770	5	aacacaattcaccggctccttagc	ctccttcattccttccatagg	4	0.667	100-500
17.	HRM 20196	6	gactcgtcactgacactgatacg	tatcattccggctccaagtctcg	3	0.324	200-400
18.	HRM 20060	6	aacaacctgagaacaccttgg	gagttatttgagaggacctaccg	3	0.439	200-500
19.	HRM 20615	6	gccaactacttcgacagcttcc	tagcccttctgacgtaatacc	3	0.498	100-600
20.	HRM 20866	7	ttgacaaaactgtagcgttgg	ctcgtaaagcctaggccatcaagc	3	0.602	150-400
21.	HRM 20948	7	aaactgagcttggctcgtttcc	cgtcagattacagtgctgcaccc	4	0.524	400-700
22.	HRM 22622	8	gatcgtctttagtcccgggtgg	aacacaattcaccggctccttagc	3	0.326	250-400
23.	HRM 23578	8	tccacacatgatcgctacatcg	aatgcctagcactcactccttgc	3	0.587	200-400
24.	HRM 23906	9	atccctagctagccttcttcc	accacctctcacgctgtacg	3	0.587	100-500
25.	HRM 24542	9	aagaacatctccttctgtgc	gctccaatcaaacgaccttcc	3	0.551	180-300
26.	HRM 25355	10	atgcatgggctaaatgatgagc	tgacagaccacaagaagtttgc	4	0.613	150-500
27.	HRM 25796	10	gcaacctgaaccacaagatagc	tgtgaatctgacttgccaatcg	5	0.755	100-400
28.	HRM 25970	11	gccacaggagacagcaagaacc	taaccgggactaaagatagagc	3	0.604	100-400
29.	HRM 27323	11	ccgcgttcacataacgatgc	aaatgggacatgttctctcg	4	0.643	200-800
30.	HRM 26213	11	ctacgtcgaaatcgtaactagc	tctccgctcggttcatctagg	4	0.684	120-800
31.	HRM 27322	11	ggccatcagtttaagtgtacg	aggccctcgcgtgtacatacc	4	0.602	200-800
32.	HRM26329	11	tttgcgtgctagggagattagc	catggccctgttctgtatgg	3	0.524	100-300
33.	HRM27183	11	tttgctaaaggagcactacgc	accaaccgggactaaagatcg	4	0.514	200-600
34.	HRM 26369	11	tgggttcgggatacaaatgc	gattactcccgttcgatagc	3	0.551	200-500
35.	HRM 28202	12	acagcgaacgtggtgtctcc	caatgcaaagtctgtacgg	3	0.605	150-220
36.	HRM 28800	12	atgaacagcaagcacatagg	gtttgcgggtaataagtgg	3	0.658	200-350
37.	HRM 27840	12	aaaggtccaatagtcatgg	gagatcgaatgctgaatgc	3	0.587	100-600
38.	HRM 28157	12	gaggtgggtgtgaccttgg	caccgattattgtcgtatgg	3	0.551	400-700
39.	HRM 28110	12	cccactgattgaccaagagc	tggaaatacagaaggagtcg	3	0.604	100-700
40.	HRM 28616	12	aatgcatgcaaacacagtgg	ccgccacctcctctatcc	3	0.524	100-400
	Total				131		
	Mean				3.28	0.542	

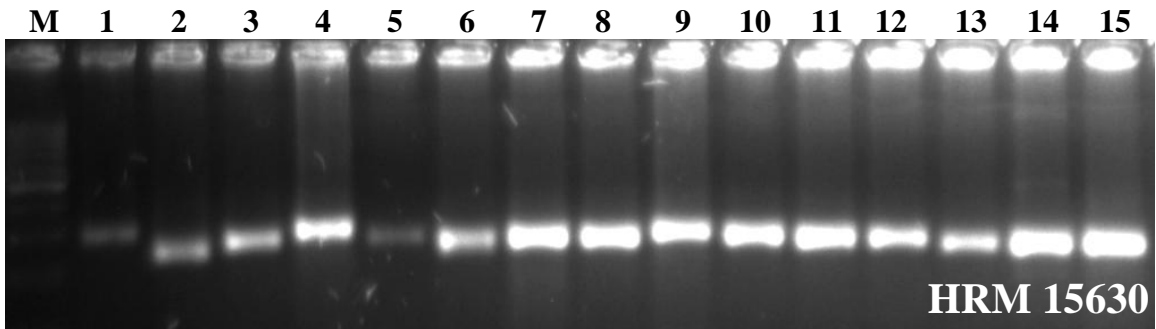
Correlation between number of bands and PIC value of 40 selected Primers = 0.350.

Table.2 Agronomic characteristics of the 30 experimental hybrids and correlation between standard heterosis for grain yield of the experimental hybrids and coefficient of marker polymorphism (CMP) based on analysis with 40 SSR markers

S.N.	Parental combination	DF	PH (cm)	PBT/P	SF (%)	GY over 100% fertility	Per day productivity (%)	Yield efficiency (kg/ha) over NDR-359	Heterosis over Better Parent	Mid Parent Heterosis	SH % over NDR-359	CMP
1.	TJ – 5769 × NDR-359	111	124	13.72	82.65	28.75	15.67	-21.92	-21.91**	-2.86	-21.91**	0.514
2.	TJ – 5769 × CSR-36	111	124	11.42	83.45	22.37	16.02	-38.74	0.83	12.33	-38.73**	0.526
3.	TJ – 5769 × PB-1	110	122	13.55	84.34	22.80	14.63	-36.81	4.00	9.66	-36.80**	0.459
4.	TJ – 64897 × NDR-359	74	106	7.21	77.27	12.80	-18.62	-66.18	-65.69**	-47.66	-65.69**	0.564
5.	TJ – 25966 × NDR-359	90	106	8.66	82.47	22.17	-6.15	-39.93	-39.92**	-16.13	-39.92**	0.500
6.	TJ – 25966 × CSR-36	83	109	8.80	85.69	19.07	-13.42	-46.30	11.13	17.27	-46.30**	0.526
7.	TJ – 25966 × PB-1	90	105	8.50	84.23	19.93	-6.84	-44.82	1.23	12.86	-44.83**	0.526
8.	TJ – 4122 × NDR-359	92	99	6.28	86.19	13.44	-4.07	-61.91	-61.91**	-44.56	-61.91*	0.500
9.	TJ – 4122 × CSR-36	95	94	6.67	81.20	13.09	-1.30	-65.07	-27.70**	-18.50	-65.06**	0.526
10.	TJ – 4122 × PB-1	95	105	6.32	84.42	14.20	-0.96	-60.60	-27.70**	-14.26	-60.59**	0.684
11.	TJ – 11010 × NDR-359	113	122	22.23	89.09	38.74	17.06	13.41	13.41**	24.71	13.41**	0.658
12.	TJ – 11010 × CSR-36	112	119	17.52	88.45	37.67	16.02	9.50	33.76**	13.90	9.52*	0.605
13.	TJ – 11010 × PB-1	113	119	21.08	89.77	38.97	17.06	14.95	40.39**	35.32	14.95**	0.658
14.	TJ – 16081 × NDR-359	107	113	19.62	85.93	35.01	11.51	-1.15	-1.14	47.03	-1.14	0.500
15.	TJ – 16081 × CSR-36	109	111	17.14	84.64	29.66	13.59	-17.52	12.09	-46.20	-17.51**	0.579
16.	TJ – 16081 × PB-1	108	112	19.35	86.43	33.14	12.21	-5.85	27.95**	13.90	-5.83	0.526
17.	TJ – 25892 × NDR-359	89	117	15.33	86.66	21.24	-7.53	-39.50	-39.48**	-18.09	-39.48**	0.579
18.	TJ – 25892 × CSR-36	87	112	14.33	84.98	22.84	-9.95	-36.21	32.03**	32.80	-36.20**	0.579
19.	TJ – 25892 × PB-1	88	119	15.67	87.73	24.42	-8.91	-29.58	29.21**	37.72	-29.58**	0.605
20.	TJ – 10365 × NDR-359	114	143	8.51	86.23	26.96	18.44	-23.60	-23.58**	-7.36	-23.58**	0.590
21.	TJ – 10365 × PB-1	112	143	8.73	87.67	26.84	16.02	-22.54	19.22**	29.68	-22.52**	0.641
22.	TJ – 39050 × CSR-36	90	108	5.60	85.37	9.63	-6.49	-79.30	-57.13**	-37.87	-79.28**	0.553
23.	TJ – 39050 × PB-1	107	107	5.54	86.55	8.80	11.17	-80.74	-64.64**	-47.11	-80.73**	0.568
24.	NDR-359 (Check)	96	105	13.39	80.00	30.43						
Correlation between CMP and standard heterosis over NDR-359 for grain yield for 23 hybrids												0.227
Correlation between CMP and per day productivity for 23 hybrids												0.122
Correlation between Grain yield and per day productivity for 23 hybrids												0.612**

**, ** significant at 5 and 1 per cent probability levels, respectively*

Fig.1 Amplification pattern of the marker HRM 15630 among the rice lines analyzed



(M.) Marker (1) Nipponbare (2.) JYOTHI (3.) TJ - 5769 (4) TJ - 64897 (5.) TJ - 64897 (6.) TJ - 11010 (7.) TJ - 16081 (8.) TJ - 16073 (9.) TJ - 10365 (10.) TJ- 4122 (11.) TJ -25892 (12.) TJ -39050 (13.) PB-1 (14.) NDR-359 (15.) CSR-36.

Fig.2 UPGMA dendrogram for the 15 genotypes based on SSR analysis using Jaccard's similarity coefficient

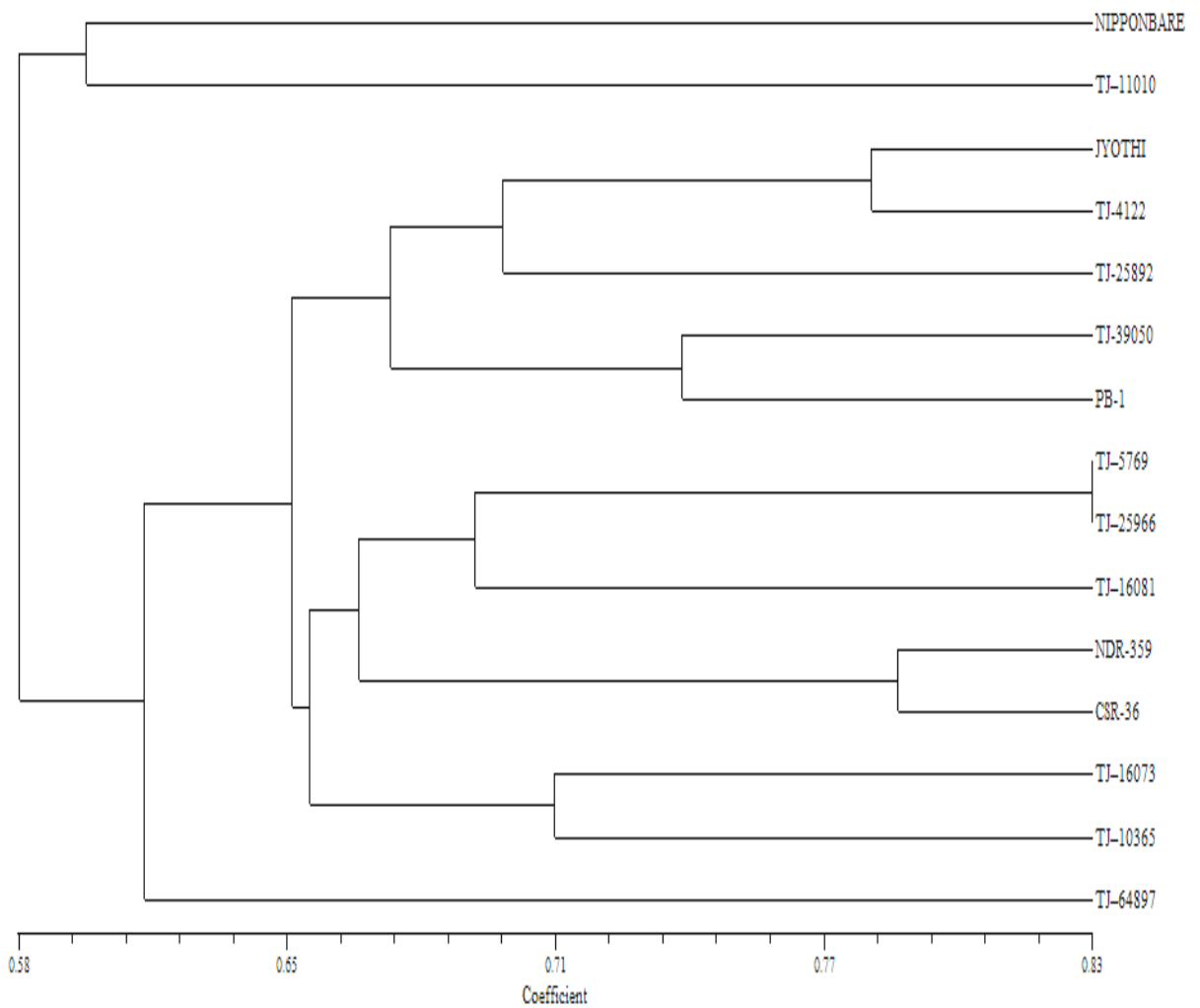
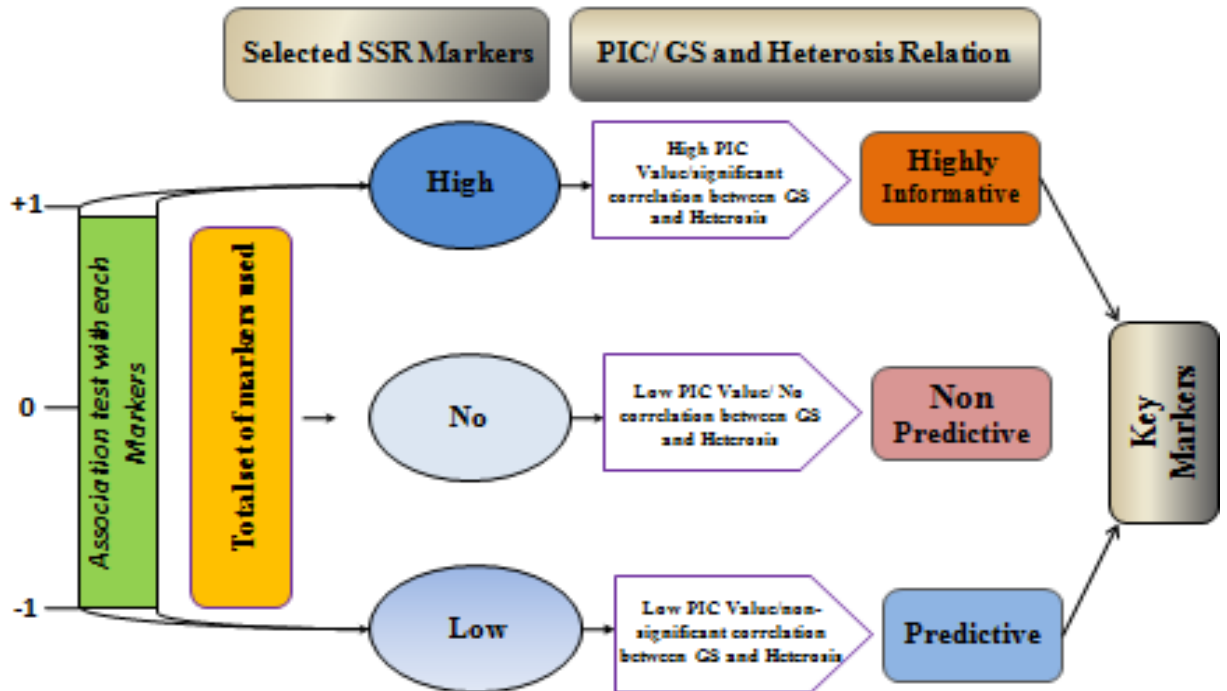


Fig.3 Development of highly informative marker concept for prediction of heterosis in rice parental lines



Rice genotypes belonging to different clusters and having a higher level of genetic diversity could be used for getting heterotic hybrids.

Correlation between CMP and yield heterosis

Coefficient of polymorphism is a measure of diversity between parental lines used for deriving the highly heterotic hybrids. The coefficient of marker polymorphism (CMP) for each hybrid was calculated based on extent of marker polymorphism among the parental lines of the test hybrid with respect to the markers analyzed. The maximum CMP value of 0.744 observed for hybrid TJ-10365 × CSR-36 showed highly negative direction of heterosis (Jaikisan 2010). The hybrid TJ-11010 × NDR-359 having CMP level of 0.658 followed by the hybrid TJ-11010 × PB-1 with CMP 0.658 and TJ-11010 × CSR-36

with 0.605 showed significant positive heterosis for grain yield. A low but positive correlation of 0.227 was observed between CMP and heterosis of grain yield respectively. Such a low value of correlation of genomic simple sequence repeats with grain yield heterosis could be due to spread across the rice genome mainly in the non-genic regions and indicating that markers selected for the present study may not be insufficient for such correlation analyses.

Therefore, need to include more markers and more hybrid combinations. The primer pairs HRM 25796, HRM 26213, HRM 28800, HRM 27323, HRM 13154 possessing a high PIC value (> 0.5) can be considered as highly informative markers for identification of parental polymorphism and its possible use in rice parental lines for predicting the heterosis (Cho *et al.*, 2004) (Fig. 3).

Correlation between CMP and per-day productivity

The per-day productivity of the thirty derived hybrid varied from 18.44 (TJ – 10365 × NDR-359) to -18.62 (TJ – 64897 × NDR-359). The hybrid TJ – 10365 × NDR-359 showed highest per day productivity percent of 18.44 followed by the hybrid TJ – 11010 × PB-1 (17.06) and TJ – 5769 × CSR-36 (16.02). The least per day productivity percent of 11.17 was recorded for the hybrid TJ – 39050 × PB-1. The yield efficiency (kg/ha) of the hybrids ranged from -80.74 (TJ – 39050 × PB-1) to 14.95 (TJ – 11010 × PB-1). The highest yield efficiency (kg/ha) was recorded for the hybrid 14.95 (TJ – 11010 × PB-1) followed by 13.41(TJ – 11010 × NDR-359) and 9.50 (TJ – 11010 × CSR-36). The lowest yield efficiency (kg/ha) was calculated for the hybrid TJ – 39050 × PB-1 (11.17). A low correlation value of 0.122 was calculated between CMP and per day productivity for 23 hybrids. The highly significant and positive correlation of 0.612 was exerted between Grain yield and per day productivity for 23 hybrids.

A large number of combinations annually made and evaluated in hybrid breeding programs, only a few hybrids are selected. This means extremely intensive labor is required, time is consumed and the selection efficiency is low. Thus, it is very important to explore alternative approaches to enhance the selection efficiency and to predict hybrid performance, such as through genomic variation of the parental lines and suitability to express higher heterosis. Aforesaid five parents TJ-11010, TJ-16081, TJ-10365, TJ-5769 and only one tester NDR-359 showing positive and significant effects for grain yield and other important traits may be utilized valuable donor parents for hybridization programme for obtaining high yield. The primers exhibiting high PIC (<0.5) were considered as highly informative key markers (Fig. 2). Similarity analysis inferred that the

parents having least similarity value can be used for achieving higher grain yield. Correlation value of 0.227 between CMP and grain yield heterosis over standard variety NDR-359 indicated that markers selected for the present study may not be sufficient for such correlation analyses and formulating informative marker concept. Therefore, need to include more markers and more hybrid combinations.

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