

Physiological and Molecular Evaluation of Positive and Negative Heterotic Rice Hybrids for Grain Yield

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ABSTRACT

Exploitation of hybrids is of greater importance to increase the productivity of rice, as it is a staple food around the world. Hybrid rice is developed by exploiting the phenomenon of heterosis. To evaluate the phenomenon of heterosis, a pot culture experiment was conducted at ICAR-Indian Institute of Rice Research, Hyderabad, during Kharif 2014. The experiment was laid out in completely randomized design with seven rice hybrids (DRRH3, DRRH2, KRH2, APMS6A X C20R, APMS6A X BCW56, APMS6A X AjayaR, PUSA5A X BR 827-35) along with their parents to evaluate whether these hybrids are positively heterotic or negatively heterotic for grain yield. Data was recorded on different parameters like SVI, root length, root volume, total biomass, stomatal conductance (gs), transpiration rate (T), panicles plant⁻¹, grain number panicle⁻¹ and yield plant⁻¹. For molecular characterization 14 hyper-variable polymorphic Expressed Sequence Tag derived Simple Sequence Repeats (EST-SSRs) primer pairs were used for PCR amplification. Of the 14 primers amplified in the hybrids, 6 are monomorphic and the rest are polymorphic. These hybrids were also analyzed for parental polymorphism wherein the coefficient of marker polymorphism was calculated and correlated to standard heterosis % with respect to grain yield. The parental combinations exhibiting CMP values of ≥ 0.7 exhibited positive heterosis and others are negatively heterotic for grain yield. It is concluded that the hybrids DRRH3, DRRH2, KRH2, APMS6A X C20R, APMS6A X BCW56 are positively heterotic and the hybrids APMS6A X AjayaR, PUSA5A X BR 827-35 are negatively heterotic.

Key words: Coefficient of Marker Polymorphism (CMP), EST-SSRs, Negative Heterosis, Positive Heterosis, Seedling Vigor Index (SVI).

INTRODUCTION

Rice (*Oryza sativa* L.) is central to the lives of billions of people and is a staple food around the world. In India, more than 65 % of the population depends on rice as their food.

Facing the challenge of ever increasing population and crop land reduction at the rate of about 10 to 35 billion hectares per year, it is obvious that the only solution for this problem is to improve the yield of rice¹¹.

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Towards realizing these goals, number of crosses involving contrasting parents are to be carried out on a continuous basis and the resulting heterosis had to be studied.

Heterosis or hybrid vigour is a phenomenon that describes the survival and performance superiority of a hybrid offspring over the average of both its genetically distinct parents. In plants, heterosis is known to be a multigenic complex trait and can be extrapolated as the sum total of many physiological and phenotypic traits including magnitude and rate of vegetative growth, flowering time, yield (in terms of panicles per plant, grain number per panicle, grain weight) and resistance to biotic and abiotic environmental rigours; each of them contributing to heterosis to a certain extent⁹. Prediction of heterosis is interesting to breeders in crops like rice and maize in which hybrids are commercially important¹⁴.

With the advent of molecular techniques, it is easy to characterize varieties with greater accuracy in a short span of time. Recently, the emphasis in crop genetics has been shifting towards a special class of SSRs called Expressed Sequence Tag derived SSRs (EST-SSRs). Since, the EST-SSRs belong to the transcribed region of the genome, they are expected to be more conserved and have a higher rate of transferability across species as compared to genomic SSRs. They are well known for high level of polymorphism and versatility and are preferred for assessment of genetic purity due to their reproducibility and amenability to automation¹⁰. This study was designed to assess the potential of EST-SSRs in the prediction of grain yield heterosis based on marker polymorphism data.

Heterosis over the mid-parent

$$\text{Per cent heterosis over mid-parent (MP)} = \frac{\text{Mean of F}_1 - \text{Mean of mid-parent}}{\text{Mean of mid-parent}} \times 100$$

Where, Mean of mid-parent = average of mean of two parents involved in F₁ i.e (P₁ + P₂)/2

Heterobeltiosis

$$\text{Per cent heterosis over better parent (BP)} = \frac{\text{Mean of F}_1 - \text{Mean of better-parent}}{\text{Mean of better-parent}} \times 100$$

MATERIAL AND METHODS

Pot culture experiment was carried out at Biotechnology Greenhouse, ICAR-IIRR, Hyderabad with seven rice hybrids which are at different heterotic levels in Completely Randomized Block Design with three replications. These hybrids include

1. DRRH-3 - APMS6A x RPHR1005
2. DRRH-2 - IR68897A x DR714-1-2R
3. KRH-2 - IR58025A x KMR3R
4. APMS6A X C20R
5. APMS6A X BCW56
6. APMS6A X AjayaR
7. Pusa5A X BR827-35

Seeds of all the genotypes were grown in separate lines in a tray. The seedlings were finally transplanted into the pots (12" diameter) after 30 days. Recommended package of agronomic practices and plant protection measures were followed as per need to raise a healthy crop. Seedling vigor index was calculated, the genotype showing the higher seed vigor index is considered to be more vigorous². Root length, root volume, total biomass was calculated. Various Photosynthetic characteristics viz., stomatal conductance (gs) and transpiration rate (T) were recorded on the adaxial surface of fully expanded leaf at 50 percent flowering by using Li-Cor 6400 portable photosynthesis measurement system (LCF Model 6400-1, LICOR, USA) between 10.00 am to 12.00 noon on the sampling date. Panicles plant⁻¹, grain number panicle⁻¹ and yield plant⁻¹ were recorded after the final harvest. The magnitude of heterosis was worked out on the basis of (i) mid-parent value, (ii) mean value of better parent.

The data obtained from pot experiments was analyzed following completely randomized block design with three replications using a statistical computer package INDOSTAT. The critical difference (CD) values were calculated at 5 percent probability level, wherever 'F' test was significant.

For EST-SSR marker analysis, leaf sample from the genotypes was collected and DNA was isolated by CTAB method⁶. Later PCR was set up and the products were resolved on agarose gel and analysed for polymorphism. The cereal EST-SSR database available online at <http://wheat.pw.usda.gov/ITMI/EST-SSR/> was used to select a set of 14 EST-SSRs that are distributed across the rice chromosomes (Table 1).

RESULTS AND DISCUSSION

Physiological traits like seedling vigor index, root length, root volume, total biomass, stomatal conductance (gs) and transpiration rate (T) and molecular analysis using EST-SSRs for polymorphism were analysed at different stages of crop and correlated to yield. Significant differences with respect to seedling vigor index were observed in all the hybrids and parents. Among the hybrids, maximum seedling vigor index was recorded in the hybrid KRH2 (1963.2) and minimum values were recorded in the hybrid APMS6A X AjayaR (1296.5). Among the parents, KMR3R recorded maximum seedling vigor (1836.2) and BR827-35 recorded minimum value of 1258.4. Significant negative heterosis and heterobeltiosis for seedling vigor index was observed in the hybrids APMS6A X BCW56, APMS6A X AjayaR and PUSA5A X BR 827-35.

Significant differences among the genotypes was found for root volume where maximum and minimum values were recorded in the hybrids KRH2 (75.0 c³) and PUSA5A X BR 827-35 (62.7 c³). Significant negative heterosis and heterobeltiosis was observed for root volume in the hybrids APMS6A X

BCW56, APMS6A X AjayaR and PUSA5A X BR 827-35. The maximum root length was recorded in the hybrid DRRH2 with value 28.3 cm followed by 28.0 cm in KRH2. Among the parents, maximum root length was recorded in PUSA5B. Significant positive heterosis was observed for root length in the hybrids DRRH2 and KRH2.

The data pertaining to total biomass recorded at 90 and 120 DAS showed significant differences. Among the parents, the maximum value for total biomass at 90 and 120 DAS was recorded in BR827-35 (16.49 g plant⁻¹) and RPHR1005 (30.40 g plant⁻¹) respectively. At 90 and 120 DAS, among the hybrids the maximum total biomass was recorded in the hybrid KRH2 (19.9 and 32.88 g plant⁻¹) and minimum values were recorded in PUSA5A X BR827-35 (14.51 and 26.73 g plant⁻¹). Negative average heterosis and heterobeltiosis were observed in the hybrid APMS6A X AjayaR (-4.49 % and -4.68 %) at 120 DAS and negative heterobeltiosis in PUSA5A X BR827-35 at 90 and 120 DAS (-12.05 %, -0.12 %).

Stomatal conductance values recorded at flowering stage varied from 0.07-0.32 mol H₂O m⁻² s⁻¹. Significant maximum and minimum values were recorded in the hybrids DRRH3 (0.32 mol H₂O m⁻² s⁻¹) and PUSA5A X BR827-35 (0.07 mol H₂O m⁻² s⁻¹) respectively. Among the parental lines maximum value was observed in APMS6B which was on par with DR 714-1-2R and KMR3R. Significant maximum and minimum values for transpiration rate were observed in the hybrids KRH2 (13.50 m mol H₂O m⁻² s⁻¹) and PUSA5A X BR827-35 (1.69 m mol H₂O m⁻² s⁻¹) respectively. Among the parental lines maximum value was observed with APMS6B (10.84 m mol H₂O m⁻² s⁻¹). Positive average heterosis and heterobeltiosis for stomatal conductance was observed only in the hybrids DRRH3 (66.25 %, 51.86 %) and KRH2 (51.86 %, 11.13 %). Negative average heterosis and heterobeltiosis was observed in rest of the

hybrids. Positive average heterosis and heterobeltiosis for transpiration rate was observed only in the hybrid checks DRRH2 (40.00 %, 20.09 %) and KRH2 (66.76 %, 32.06 %). Negative average heterosis and heterobeltiosis was observed in rest of the hybrids.

Number of panicles plant⁻¹ varied from 17.3 to 27.0 among all the lines. Significant maximum and minimum values for panicles plant⁻¹ were observed in the hybrids KRH2 (27.0) and PUSA5A X BR827-35 (17.3) respectively. Among the parents, maximum and minimum panicle number was recorded in IR68897B (24.7) and AjayaR (18.3) respectively. Positive average heterosis for number of panicles was observed in all the hybrids except in PUSA5A X BR 827-35 (-15.45 %). Similar results for positive heterosis and heterobeltiosis for number of panicles were reported by Joshi⁸ and Pham *et al*¹².

Significant difference for grain number was observed between the parents and hybrids. The maximum and minimum grain number were recorded in the hybrid APMS6A X BCW56 (291.3) which was on par with APMS6A X C20R and in APMS6A X AjayaR (222.7) respectively. Similar results for grain number were reported by Bhave *et al*⁴. Significant positive heterosis and heterobeltiosis was observed in all the hybrids for grain number panicle⁻¹. Significant positive heterosis for grains per panicle has been reported by Suresh *et al*¹³.

The average values for grain yield plant⁻¹ varied from 14.1 to 34.9 g plant⁻¹. Among the parents, the maximum and minimum grain yield was recorded in KMR3R (27.6 g) and in AjayaR (20.3 g) respectively. The maximum and minimum grain yield among the hybrids was recorded in APMS6A X BCW56 (34.9 g) and PUSA5A X BR 827-35 (14.1 g) respectively. Significant seed yield with similar results were reported by Arasakesary *et al*³. Among the seven hybrids, positive average heterosis and heterobeltiosis

for grain yield was observed with five crosses namely DRRH3, DRRH2, KRH2, APMS6A X C20R and APMS6A X BCW56. Positive heterosis for grain yield was earlier reported by Dalvi and Patel⁵. Negative average heterosis and heterobeltiosis was observed in the hybrids APMS6A X AjayaR (-35.98 %, -40.83 %) and PUSA5A X BR827-35 (-40.06 %, -40.14 %). Similar results were reported by Joshi⁸ and Abd Allah¹.

The 14 EST-SSR markers (table 1.) were also analyzed for parental polymorphism. The number of fragments amplified by each primer in the parental lines and their coefficient of marker polymorphism is calculated and presented in table 4. The number of alleles amplified by each primer pair ranged from 1-3 in the parents and the fragments amplified by the primer RMES 7-1 in parents is presented in figure 1.

The hybrids were also analyzed for polymorphism using these EST-SSR markers (Table 1). The number of alleles amplified by each primer pair ranged from 1-4 in the hybrids. The marker RMES 7-1 amplified a maximum number of four alleles (Fig 2), while the markers, RMES 8-1 (Fig 4), RMES 9-2 (Fig 5), RMES 12-2 (Fig 7) exhibited amplification of three polymorphic alleles. RMES 7-2 (Fig 3) and RMES 10-2 (Fig 6) amplified two polymorphic alleles.

The coefficient of marker polymorphism and standard heterosis % for different hybrids is mentioned in table 5. Using these markers, a higher positive correlation ($r = 0.79$) was observed between the CMP value among parental lines with standard heterosis of 7 experimental hybrids. Clearly, those parental combinations exhibiting CMP values of ≥ 0.7 exhibited positive heterosis and those with low CMP values (≤ 0.5) exhibited negative heterosis for grain yield. Similar results were reported by Jaikishan *et al*⁷, in rice hybrids with positive correlation between standard heterosis and CMP values.

Table 1: List of EST-SSR markers used in the study

S.No	Marker	Chrom No.	Forward Sequence	Reverse Sequence	Putative Function
1	RMES 7-1	7	CCTCTCTCCCTCTGTCTC	CCACGTAGGAGGCAACATC	<i>O. sativa</i> (japonica) homeodomain leucine zipper protein 14 mRNA
2	RMES 7-2	7	TGGCCCTCATGAGACATACA	TTAAGCAATCAAAGGGGGTG	<i>O. sativa</i> (japonica), mRNA
3	RMES 8-1	8	GGAGGAGGAGGAGGATCTTG	TTCTCCGACGACGAGTTCT	<i>O. sativa</i> (japonica), mRNA encoding a conserved protein with hypothetical function
4	RMES 8-2	8	GGATCGATGACGGACAGAAT	GAGATGAGATTGCAACACGG	<i>O. sativa</i> (japonica), mRNA
5	RMES 9-1	9	CTGGAGATCGATCGAGAGGT	TAGCCAAGGAGACTGCCTGT	<i>O. sativa</i> precursor microRNA 169h gene, complete sequence
6	RMES 9-2	9	CCACGTTGATAAGCTCATTGC	TGGGCACCGAAAATAAAAATC	<i>O. sativa</i> (japonica) cDNA clone:002-102-A07, full insert sequence encoding a protein kinase domain containing protein
7	RMES 10-1	10	CAGGGGTACGTCTACAAGG	TATACCCGGCGAAATACGTC	<i>O. sativa</i> (japonica) gene encoding a putative nitrate transporter (OSJNB001411.9) mRNA
8	RMES10-2	10	AACAGTTCCAACAGAACCCG	TTATTGCTGATGCTGCGAC	
9	RMES 11-1	11	TTGACCTAGCCAACCTCACC	CGGAGTACAGTCCACCAC	DHHC zinc finger domain, putative
10	RMES 11-2	11	GGTGGACTACTGCAGCACTG	TCTTGGTCGGAAACAGCTCC	
11	RMES 12-1	12	ACGACGTCTGTCTGCTCTT	ACTTGTACTGCCGTGGTTC	
12	RMES 12-2	12	TGTGTTCACTGCTGCTTCG	CGTGTTCAGAAAGTTGCAGT	YABBY protein
13	ESSR 12-1.5	12	TTCTCTCTTCTTCTCCGC	GTTGAGGTACGCGAAGATCC	
14	ESSR12-20.2	12	GGTGTTCAGGGCTCTACT	TCATGGAATGGAACAACCA	

Table 2: Seedling vigor index, Root volume (c³), Root length (cm), Total biomass (g plant⁻¹), Stomatal conductance (mol H₂O m⁻² s⁻¹), Transpiration rate (m mol H₂O m⁻² s⁻¹), Panicles plant⁻¹, Grain number plant⁻¹, Yield (g plant⁻¹) in rice hybrids and their parents

S.No	Parents / Hybrids	Seedling vigor index	Root Volume (c ³)	Root length (cm)	Total biomass (g plant ⁻¹)		Stomatal conductance (mol H ₂ O m ⁻² s ⁻¹)	Transpiration rate (m mol H ₂ O m ⁻² s ⁻¹)	Panicles plant ⁻¹	Grain number panicle ⁻¹	Yield (g plant ⁻¹)
					90 DAS	120 DAS					
1	APMS6B	1771.3	72.0	26.7	14.36	27.97	0.26	10.84	19.33	169.7	24.0
2	IR68897B	1586.7	52.3	25.5	12.65	22.66	0.14	6.35	24.67	168.7	25.5
3	IR58025B	1422.5	78.3	23.8	15.39	26.65	0.11	5.97	21.33	182.3	24.8
4	PUSA5B	1401.3	72.0	26.9	11.29	20.78	0.09	3.33	18.67	182.3	23.5
5	RPHR1005	1674.3	58.3	24.3	12.94	30.40	0.13	7.76	20.33	177.0	25.4
6	DR714-1-2R	1675.2	71.7	22.2	10.84	22.48	0.25	8.88	23.00	188.7	20.9
7	KMR3R	1836.2	70.3	25.9	13.54	23.64	0.24	10.22	20.67	191.0	27.6
8	C20R	1302.8	61.7	25.2	12.87	23.47	0.13	3.88	22.67	170.3	23.4
9	BCW56	1290.9	69.0	23.4	14.80	27.65	0.16	4.85	23.67	168.0	23.2
10	AjayaR	1375.3	67.7	24.5	15.79	28.08	0.08	2.97	18.33	182.7	20.3
11	BR827-35	1258.4	75.3	25.9	16.49	26.77	0.11	3.25	22.33	184.0	23.5
12	DRRH3	1892.0	74.0	24.5	16.33	32.08	0.32	7.53	23.67	240.3	31.9
13	DRRH2	1854.5	71.7	28.3	17.04	28.69	0.16	10.66	24.00	235.7	31.0
14	KRH2	1963.2	75.0	28.0	19.90	32.88	0.27	13.50	27.00	249.7	33.3
15	APMS6A X C20R	1638.5	68.3	23.6	16.99	31.03	0.09	3.02	22.67	288.0	31.4
16	APMS6A X BCW56	1485.1	68.0	24.5	16.89	30.00	0.13	3.70	23.00	291.3	34.9
17	APMS6A X AjayaR	1296.5	65.7	21.9	15.82	26.77	0.09	2.97	21.67	222.7	14.2
18	PUSA5A X BR827-35	1319.3	62.7	23.4	14.51	26.73	0.07	1.69	17.33	242.7	14.1
	Mean	1558.0	68.6	24.9	14.91	27.15	0.16	6.19	21.91	207.5	25.2
	SEm	12.876	2.494	0.737	0.68	0.969	0.003	0.142	0.594	5.012	0.987
	CD (p=0.05)	37.312	7.184	2.127	1.959	2.79	0.01	0.41	1.714	14.433	2.848

Table 3: Average heterosis (Ht) and Heterobeltiosis (Htb) estimates for Seedling vigor index, Root volume, Root length, Total biomass, Stomatal conductance, Transpiration rate, Panicles plant⁻¹, Grain number plant⁻¹, Yield in rice hybrids

S.No	Hybrid checks / Hybrids	Seedling vigor index		Root Volume		Root length		Total Biomass			
		Ht	Htb	Ht	Htb	Ht	Htb	At 90 DAS		At 120 DAS	
								Ht	Htb	Ht	Htb
1	DRRH3	9.82	6.81	13.55	2.78	-3.66	-8.00	19.63	13.75	9.93	5.54
2	DRRH2	13.71	10.70	15.59	0.00	18.60	10.99	45.04	34.67	27.11	26.59
3	KRH2	20.49	6.91	0.90	-4.26	12.62	7.98	37.55	29.26	30.74	23.36
4	APMS6A X C20R	6.60	-7.50	2.24	-5.09	-8.81	-11.38	24.76	18.32	20.66	10.95
5	APMS6A X BCW56	-3.00	-16.16	-3.55	-5.56	-2.20	-8.25	15.84	14.10	7.88	7.26
6	APMS6A X AjayaR	-17.59	-26.81	-5.97	-8.80	-14.21	-17.75	4.97	0.21	-4.49	-4.68
7	PUSA5A X BR827-35	-0.79	-5.85	-14.93	-16.81	-11.42	-13.12	4.44	-12.05	12.46	-0.12

S.No	Hybrid checks / Hybrids	Stomatal conductance		Transpiration rate		Panicles plant ⁻¹		Grain number panicle ⁻¹		Yield	
		Ht	Htb	Ht	Htb	Ht	Htb	Ht	Htb	Ht	Htb
1	DRRH3	66.25	22.93	-19.09	-30.58	19.33	16.39	38.65	35.78	29.02	25.29
2	DRRH2	-19.28	-36.14	40.00	20.09	0.70	-2.70	31.90	24.91	33.68	21.71
3	KRH2	51.86	11.13	66.76	32.06	28.57	26.56	33.75	30.72	27.06	20.63
4	APMS6A X C20R	-52.50	-64.63	-58.93	-72.11	7.94	0.00	69.41	69.08	32.80	31.15
5	APMS6A X BCW56	-39.11	-50.47	-52.88	-65.90	6.98	-2.82	72.56	71.71	48.08	45.62
6	APMS6A X AjayaR	-48.41	-66.64	-56.92	-72.57	15.04	12.07	26.40	21.90	-35.98	-40.83
7	PUSA5A X BR827-35	-29.67	-35.69	-48.68	-49.32	-15.45	-22.39	32.48	31.88	-40.06	-40.14

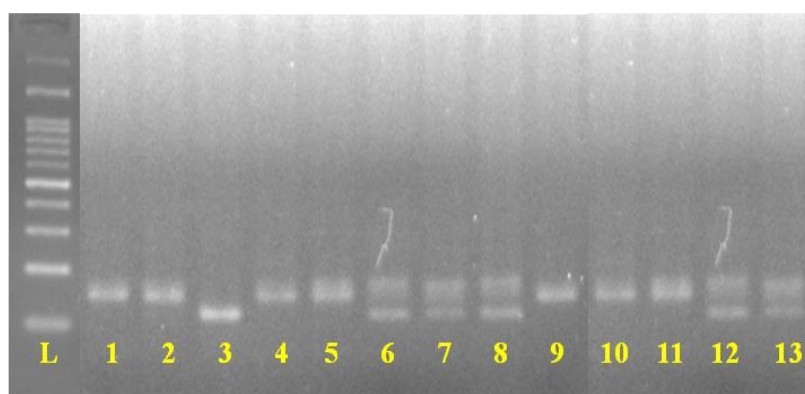
Table 4: Number of fragments amplified by EST-SSR markers and coefficient of marker polymorphism in the hybrids

S. No	EST SSR Marker	APMS 6A X C20R		P/M	APMS6A X BCW56		P/M	APMS6A X AjayaR		P/M	PUSA5A X BR 827-35		P/M	DRRH3		P/M	KRH2		P/M	DRRH2		P/M
		APMS6B	C20R		APMS6B	BCW56		APMS6B	AjayaR		PUSA5B	BR 827-35		APMS6B	RPHR 1005		IR58025B	KMR3R		IR68897B	DR 714-1-2R	
1	RMES 7-1	1	1	M	1	1	M	1	1	M	2	2	M	1	2	P	1	2	P	1	2	P
2	RMES 7-2	2	1	P	2	1	P	2	3	P	2	2	M	2	3	P	2	2	M	2	2	M
3	RMES 8-1	3	2	P	2	3	P	2	2	M	1	1	M	2	2	M	3	2	P	2	3	P
4	RMES 8-2	1	1	P	1	3	P	1	1	M	1	2	P	1	1	M	3	3	M	3	2	P
5	RMES 9-1	1	1	M	1	3	P	1	2	P	1	2	P	1	2	P	1	3	P	1	2	P
6	RMES 9-2	1	2	P	1	2	P	1	2	P	2	1	P	1	2	P	1	2	P	1	2	P
7	RMES 10-1	3	1	P	2	2	M	3	1	P	3	2	P	3	1	P	3	1	P	3	2	P
8	RMES 10-2	2	2	M	3	1	P	3	2	P	2	2	M	3	2	P	3	1	P	1	1	M
9	RMES 11-1	1	1	M	3	2	P	2	2	M	1	1	M	1	1	M	2	2	M	1	2	P
10	RMES 11-2	2	1	P	1	1	M	1	1	M	2	2	M	2	1	P	2	1	P	1	1	M
11	RMES 12-1	1	2	P	1	2	P	2	2	M	3	2	P	2	2	M	2	2	M	2	1	P
12	RMES 12-2	2	2	M	2	3	P	1	2	P	1	1	M	2	1	P	1	2	P	1	3	P
13	ESSR 12.1.5	3	1	P	1	2	P	2	2	M	2	1	P	3	1	P	2	3	P	2	2	M
14	ESSR 12.20.2	1	1	M	1	1	M	1	2	P	1	1	M	1	2	P	1	2	P	1	2	P
	CMP			0.6			0.7			0.4			0.4			0.7			0.7			0.7

Where P- Polymorphic and M-Monomorphic, CMP- Coefficient of Marker Polymorphism

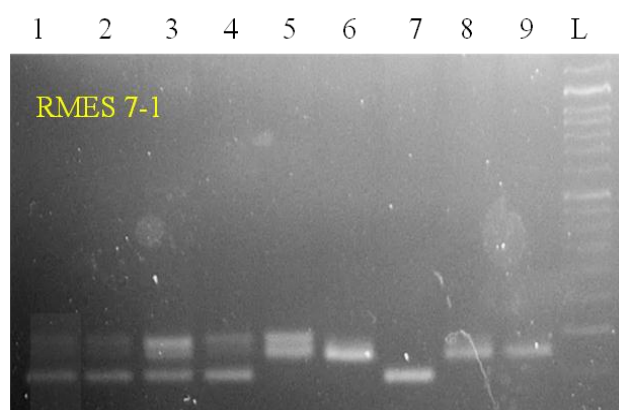
Table 5: Correlation between standard heterosis for total grain yield of 7 test hybrids and coefficient of marker polymorphism (CMP) for 14 EST-SSRs

Cross	A line	R line	Grain yield (Kg/ha)	standard heterosis %	CMP	Correlation
APMS6A X C20R	APMS6B	C20R	4721	2.6	0.6	0.71278*
APMS6A X BCW56	APMS6B	BCW56	4739	3.0	0.7	
APMS6A X AjayaR	APMS6B	AjayaR	4103	-10.8	0.4	
PUSA5A X BR827-35	PUSA5B	BR827-35	4158	-9.6	0.4	
DRRH3	APMS6B	RPHR1005	4834	5.1	0.7	
KRH2	IR58025B	KMR3R	5948	29.3	0.7	
DRRH2	IR68897B	DR714-1-2R	4679	1.7	0.7	
Standard check (Jaya)			4600			

* Significant at $P < 0.0$ **Fig 1. Amplification pattern of EST-SSR marker RMES 7-1 in rice parental lines**

L-Ladder, 1-APMS6B, 2-IR68897B, 3-IR58025B, 4-PUSA5B, 5-RPHR1005, 6-DR714-1-2R, 7-KMR3R, 8-IL 50-10, 9-C20R, 10-BCW56, 11-AjayaR, 12-IR66, 13-BR 827-35

1- APMS6A X IL 50-10
 2-APMS6A X C20R
 3-APMS6A X BCW56
 4-APMS6A X AjayaR
 5-PUSA 5A X IR 66
 6-PUSA 5A X BR 827-35
 7-DRRH3
 8-KRH2
 9-DRRH2
 L- 100 bp Ladder

**Fig 2. Amplification pattern of EST-SSR marker RMES 7-1 in rice hybrids**

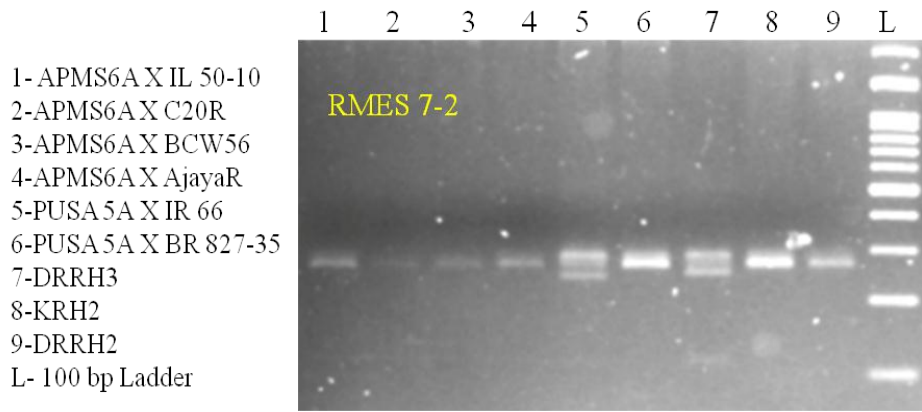


Fig 3. Amplification pattern of EST-SSR marker RMES 7-2 in rice hybrids

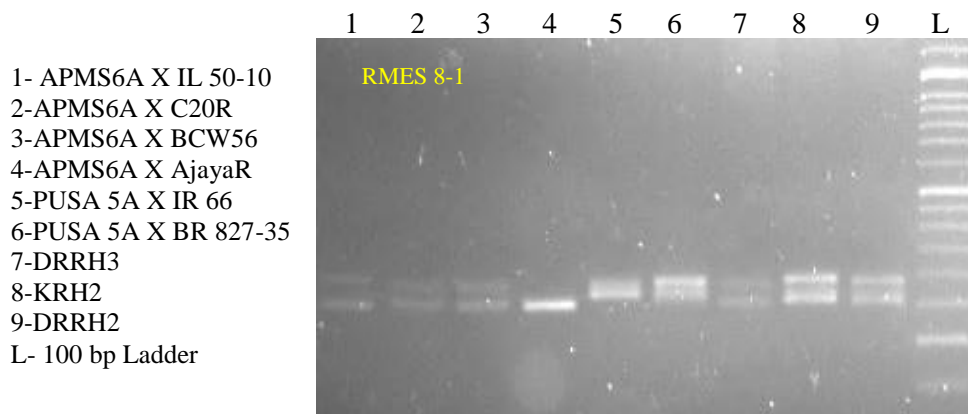


Fig 4. Amplification pattern of EST-SSR marker RMES 8-1 in rice hybrids

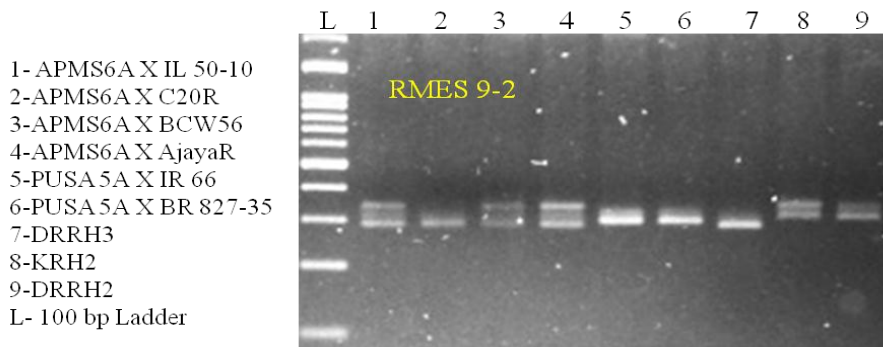


Fig 5. Amplification pattern of EST-SSR marker RMES 9-2 in rice hybrids

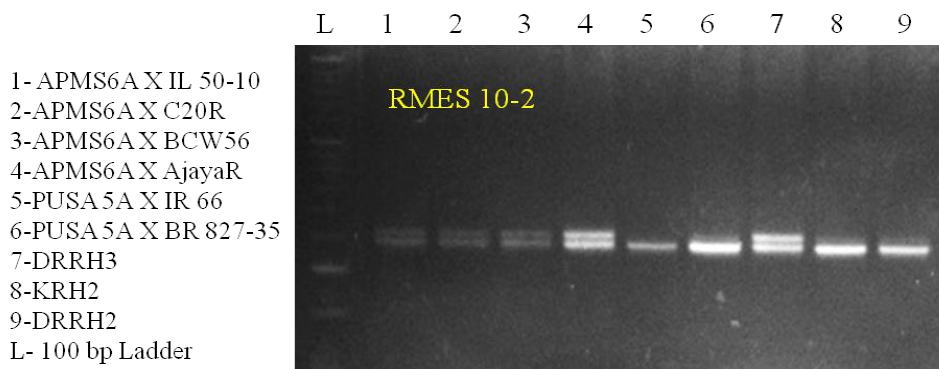


Fig 6. Amplification pattern of EST-SSR marker RMES 10-2 in rice hybrids

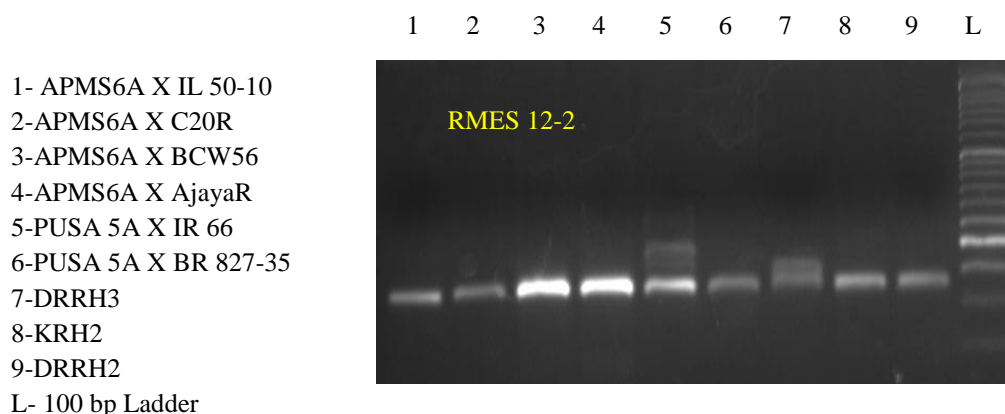


Fig 7. Amplification pattern of EST-SSR marker RMES 12-2 in rice hybrids

CONCLUSION

Both the physiological and molecular experimental results concluded that the hybrids DRRH3, DRRH2, KRH2, APMS6A X C20R, APMS6A X BCW56 are positively heterotic and the hybrids APMS6A X AjayaR, PUSA5A X BR 827-35 are negatively heterotic for grain yield.

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