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Molecular marker based estimates of genetic distance and prediction of heterosis in rice (*Oryza sativa*)

AMIT KUMAR[1], VIKRAM JEET SINGH[2], GOPALA KRISHNAN S[3], PROLAY K BHOWMICK[4], K K VINOD[5], RAKESH SETH[6], M NAGARAJAN[7], R K ELLUR[8], HARITHA BOLLINEDI[9] and ASHOK KUMAR SINGH[10]

ICAR-Indian Agricultural Research Institute, Pusa, New Delhi 110 012, India

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ABSTRACT

The association of parental divergence based on simple sequence repeats (SSR) markers and heterosis in 96 rice (*Oryza sativa* L) hybrids was investigated for yield and component traits under four environments. Hybrids were derived from four cytoplasmic male sterile (CMS) lines and 24 iso-cytoplasmic restorers (ICR). The genetic distance (GD) and heterosis were significantly correlated for the number of productive tillers per plant (0.537) and panicle length (0.386) in Delhi under early sowing. Under late sowing, negative correlations were also observed (-0.435, -0.401) with pollen fertility percentage. The GD and heterotic performance of hybrids were significantly correlated for panicle length (r=0.35) and number of effective tillers per plant (r=0.51). At Karnal, significant association between GD and hybrid grain yield (0.615) was observed. Besides, insignificant correlations were observed for some other traits at different locations. The non-significant correlations indicated the need of employing trait related functional/ gene based markers as well as using more abundant markers for accurately predicting the hybrid performance.

Key words: Correlation, Genetic distance, Heterosis, Prediction, Molecular markers, Rice

Development of heterotic hybrids is one of the key objectives in rice breeding to sustain food security of India. In rice (*Oryza sativa* L), wild abortive cytoplasmic male sterility (WA-CMS) system has been standardized for hybrid development, using the three-lines, male steriles (A), maintainers (B) lines and restorers (R). To harness maximum heterosis, isogenic A and B lines are maintained genetically diverse from the R lines (Krishnan *et al.* 2012). Under this system, prediction of heterosis has been a challenge to plant breeders, owing to several complexities such as parental kinship, geographic origin and morphological variations. Heterosis prediction uses several parameters such as *per se* performance, combining ability and genetic diversity. However, estimation of combining ability and hybrid performance requires elaborate,time consuming and expensive experimentation, usingtestcross hybrids derived using selected parental lines. Therefore, hybrid evaluations are often limited to fewer parents, and hybrids. Breeders are in search of alternative, cost-effective and resource efficient approaches that facilitate accelerated hybrid development. For achieving this in hybrid rice development, restorer diversification has been suggested as a plausible approach (Zhang *et al.* 2010; Krishnan *et al.* 2013). Molecular diversity, particularly ensuing from the derivatives of diverse commercial hybrids could lead to restorer diversification (Kumar *et al*. 2019), and may offer options to select better combiners.

Iso-cytoplasmic restorers (ICR) are derivatives of WA-CMS rice hybrids, that carry WA cytoplasm and homozygous set of restorer genes (Kumar *et al.* 2019a, b), *Rf3* and/or*Rf4* (Shidenur *et al.* 2019). In our earlier study, a set of 390 ICRs were selected from segregating population of 25 diverse and popular commercial hybrids (Kumar *et al.* 2017a), which were further reduced to a subset of 100 ICRsbased on agronomic performance and characterized for the presence of *Rf3* and *Rf4*genes (Kumar *et al.* 2017b). In the present study, 25 of these ICRs were assessed for therelationship between microsatellite (SSR) marker-based genetic diversity and the heterotic performance of hybrids derived from them.

MATERIALS AND METHODS

Plant materials and field experiments

Twenty-five ICR lines (listed in Table 3) in F_6

Present address: [1]Scientist (amit4118@gmail.com), ICAR RC for NEH Region, Umiam 793 103, Meghalaya; [2]Senior Research Fellow (jeet2012vikram@gmail.com); [3]Principal Scientist (krish.icar@gmail.com); [4]Scientist (prolaybhowmick@gmail.com); [5]Principal Scientist (kkvinodh@gmail.com); [6]Principal Scientist (rseth101@), Regional Station, Karnal 132 001; [7]Principal Scientist, Rice Breeding and Genetics Research Centre, Aduthurai 612 101, Tamil Nadu; [10]Joint Director (Research) and Head (aks_gene@yahoo.com)

generation were crossed with four male sterile lines namely IR 79156A, IR 58025A, Pusa 6A and RTN 12A resulting in 100 hybrids. The 10-15 numbers of panicles were used for each cross to ensure sufficient quantity of seeds (>400) for multi-locational evaluation. Testcross hybrids were evaluated during *kharif* 2015 at three locations namely, Delhi, Karnal and Pusa.The experiment was laid out in Augmented RBD with four blocks and 10 checks. The 21 days old seedlings were transplanted with one seedling per hill at spacing of 20 × 15cm.

Table 1 Genetic variation for various agro-morphological traits in the hybrids derived from iso-cytoplasmic rice restorers

Trait	Mean	Min	Max	Median	SD	CV
PH	93.06	67.89	121.00	92.94	9.53	10.24
NT	17.61	6.53	48.60	17.05	2.82	15.99
PL	25.49	5.31	30.51	25.59	2.42	9.50
FG	127.66	10.91	244.13	126.93	22.02	17.25
UFG	55.83	6.47	139.24	55.17	19.94	35.71
SF	69.43	6.53	99.67	70.31	12.58	18.12
TW	20.16	7.20	124.55	19.52	4.22	20.93
FP	105.78	22.54	296.15	103.13	27.73	26.21
SP	23.75	2.56	255.21	19.69	9.91	41.74
PF	77.57	1.77	97.18	84.89	13.74	17.72
DFF	93.70	75.28	115.63	93.28	7.92	8.45
YPP	29.90	10.70	71.53	28.22	6.10	20.39

PH, Plant height; NT, number of tillers; PL, panicle length; FG, number of fertile grains per panicle; SF, Per cent spikelet fertility; TW, test weight; FP, number of fertile pollen per microscopic field; DFF, days to fifty percent flowering; YPP, yield per plant.

Evaluation of agro-morphological traits

Five uniform looking healthy plants per hybrid was observed for traits namely days to 50% flowering (DFF), plant height (PH), panicle length (PL), tiller number (NT), grains per panicle (FG), pollen fertility (PF), spikelet fertility (SF), test weight (TW) and yield per plant (YPP) at all three locations. Post-harvest data were also measured form the same plants. At anthesis, three randomly picked spikelets from different positions on the panicle were split open to collect anthers which were assessed for pollen sterility using standard protocol (IRRI 2013; Kumar *et al.* 2018).

Molecular marker analysis

A 50 genome-wide SSR markers of generation challenge programme (GCP) panel were used for genotyping the parents. The DNA extraction and polymerase chain reaction were carried out as described in

Table 2 Mid parental and restorer parental heterosis among the hybrids in the multi-location evaluation

Variable	Location	HoRP (%)					HoMP* (%)				
		Mean	Median	Min	Max	SD	Mean	Median	Min	Max	SD
NT	Delhi (Timely sown)	21.02	18.66	-43.91	172.61	34.14	13.36	11.72	-47.48	144.08	28.13
PL		6.06	5.60	-14.01	36.24	8.50	6.09	6.00	-11.73	26.90	6.13
SF		-6.52	-5.44	-67.45	47.48	18.28	-10.16	-8.50	-68.42	24.56	14.27
PF		-8.67	-5.51	-92.05	48.15	21.88	-11.35	-6.66	-92.35	15.71	19.36
YPP		34.44	29.70	-64.01	155.98	45.01	33.03	34.71	-49.12	137.60	38.01
NT	Delhi (Late sown)	20.45	14.04	-24.26	133.67	29.90	20.99	14.20	-19.59	104.92	27.93
PL		2.92	2.27	-22.19	29.19	9.63	3.95	3.32	-15.83	19.46	7.30
SF		-4.04	-3.78	-69.53	40.71	18.78	-2.33	-2.74	-69.12	36.22	16.69
PF		24.45	-1.22	-98.28	483.59	131.55	-9.12	-2.72	-98.31	76.32	36.36
YPP		23.25	15.30	-60.37	252.60	48.76	33.29	23.75	-49.79	229.39	46.05
NT	Pusa, Bihar	28.08	22.13	-59.38	141.03	39.87	2.74	2.60	-62.42	70.14	26.28
PL		4.12	0.89	-33.34	47.04	15.76	6.69	6.11	-24.33	29.55	11.42
SF		19.93	12.74	-89.63	299.27	51.00	14.39	13.93	-90.16	117.46	27.98
PF		-2.30	-0.34	-94.19	125.95	29.64	-4.67	-0.04	-94.48	58.12	23.02
YPP		15.53	8.72	-68.43	157.80	38.50	18.85	15.82	-65.09	111.13	29.21
NT	Karnal	0.84	-1.04	-46.56	59.05	20.71	-3.84	-5.56	-41.43	55.48	17.67
PL		8.01	6.16	-87.32	161.99	25.91	10.35	9.76	-7.67	27.95	7.22
SF		-2.91	-7.53	-37.58	111.13	23.07	-6.25	-6.00	-42.99	22.48	11.76
PF		8.89	0.98	-98.55	185.49	52.11	-6.79	-3.39	-98.66	91.49	37.01
YPP		9.70	6.48	-44.48	111.14	28.83	21.89	11.40	-33.18	178.24	36.10

*Mid parent values are calculated based on the agronomic performance of corresponding maintainer lines for the CMS parents. HoRP, heterosis over restorer parent; HoMP, heterosis over mid parent; NT, number of tillers; PL, panicle length; SF, per cent spikelet fertility; PF, per cent pollen fertility; YPP, yield per plant

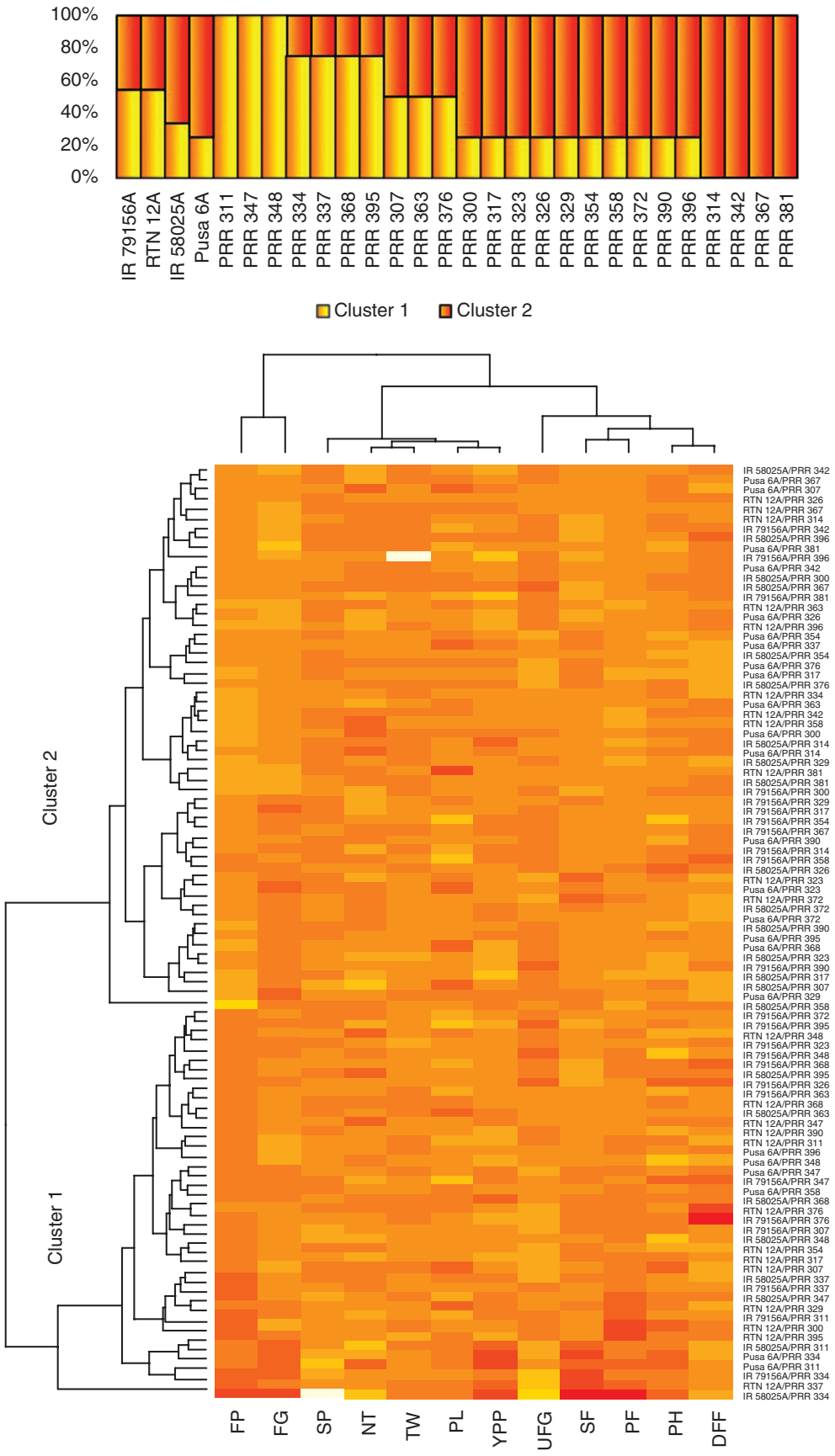

Fig 1 Hierarchical agglomerative clustering and heatmap of hybrids derived from iso-cytoplasmic restorers based on agromorphology. The bar diagram on top indicates the proportion of hybrids derived from each of the parents that have got grouped in to two clusters.

Kumar *et al*. (2017b).

Statistics and analysis

Four hybrids derived from PRR 386 were dropped from analyses due to poor performance. The agronomic performance of hybrids was subjected to analysis of variance and phenotypic diversity. Hierarchical agglomerative clustering using Euclidean distances was performed and the heatmap was generated using the R function 'heatmap'. The standard heterosis was computed, and correlated with the GD among the parents, based on Nei's distance computed from SSR data.

RESULTS AND DISCUSSION

The reason for this unreliability could be the random diversity estimates that might involve several genetic loci that are not associated with heterosis. Therefore, it would be wise to estimate GD from those specific loci (heterotic loci) that might be associated with heterosis for its prediction. Hybrid development would be successful when heterotic heterozygosity associated with agronomic yield is brought in among parental lines (Zhang *et al.* 1995).

Although several previous investigations examined the relation between GD and heterosis, an apparent relationship still remains elusive. The ambiguity could be attributed to methods of estimating GD to its random estimates. Further, evaluation of combining ability was also used for parental line selection.

A*gro-morphological and molecular variation present among the parental lines*

The ICRs showed wide range of variability for all the morpho-agronomical traits studied. Pollen and spikelet fertility ranged at fertile range indicating that *Rf* genes have little background interaction (Kumar *et al.* 2019). Yield per plant indicated that ICRs possessed good *per se* performance. Molecular diversity analysis revealed 33 polymorphic markers out of 50 (66%), and produced 84 alleles with an average of 2.54 allele/marker. Polymorphism information content ranged from 0.06 to 0.60 with an average of 0.296. This indicated a moderate genetic diversity among the parents.

Genetic divergence among the lines

Morphology data divided the parental lines into two clusters, cluster A and cluster B. Cluster A had two sub-clusters, while cluster B had three. Both ICRs and parents were found distributed in both the clusters. GD between parents ranged from 0.11 to 0.44, with an average of 0.235 (Table 1). The widest GD (0.44) was between IR 58025A and PRR 314 followed by Pusa 6B and PRR 314 (0.43), whereas it was smallest (0.11) between Pusa 6B and PRR 376.The estimates of GD also indicated a moderate divergence diverse, suggesting they could be useful in hybrid development. Two major clusters, Cluster 1 with two sub-cluster sand Cluster 2 with four sub-clusters were detected using molecular data also. Except three restorers, DRR 714, RPHR 1005 and PRR 78, all the remaining lines were found distributed among the clusters.

Phenotypic diversity among the hybrids

The hybrids showed two distinct clustering based on morphological diversity (Fig 1). The first cluster had 39 hybrids (40.6%) and the second had 57 hybrids (59.4%). ICRs had more distinct effect on clustering then the female parents. Three ICRs, PRR311, PRR347 and PRR348 had all of their hybrids grouped into cluster 1, while four of them, PRR314, PRR342, PRR367 and PRR381 had all of their hybrids falling in Cluster II. Cluster I and II had prominent membership of hybrids derived from an additional set of four and 10 ICRs, respectively.

Heterosis analysis and correlation between genetic distance and heterosis

Level of heterosis in hybrids varied between traits and locations indicating strong influence of environment. The heterosis over the restorer parent for number of effective tillers per plant (NT) ranged from -59.38% (Pusa, Bihar) to 172.6 % (under timely sown conditions of Delhi), while the mid-parental heterosis ranged between -62.4% (Bihar) and 144.1%, under timely sown conditions at Delhi (Table 2).Similarly, heterosis for spikelet fertility over the restorer parent was the lowest (-89.6%) as well as the highest (299.3%) under Pusa, Bihar conditions. The mid-parent heterosis for spikelet fertility ranged from -90.2% to 117.5% under Pusa, Bihar conditions among all the other locations. Maximum restorer parent heterosis was recorded at Delhi under late sown conditions (252.6%) for grain yield per plant (YPP), whereas the minimum was recorded at Pusa, Bihar (-68.43%). Location-wise, mid-parental heterosis for YPP was maximum at Delhi under late sown conditions (229.39%), while it was lowest under Pusa, Bihar (-65.09%).

Correlation analysis between GD and heterosis were calculated and presented in Table 3.Under timely sown conditions of Delhi, positive significant correlation was present with number of tillers (0.537, RTN 12A) and pollen fertility percentage (0.441, IR 58025A). In Karnal, yield per plant had significant association for RTN 12A (0.615). The better parent heterosis for number of effective tillers per plant (NT) ranged from -59.38% (Bihar) to 172.61 % (timely sown conditions of Delhi), with an average of 70.39%; spikelet fertility percentage had shown a variation ranging from -89.63% to 299.77 (both at Bihar) with a mean heterosis of 6.46%. As far as yield per plant is considered, minimum heterosis was found in Bihar (-68.43%), whereas

Table 3 Genetic distance between the parental lines of hybrids derived using four cytoplasmic male sterile lines and 25 iso-cytoplasmic restorers

ICR parents	Male sterile parents			
	IR 58025B	IR 79156B	Pusa 6B	RTN 12B
PRR 300	0.20	0.19	0.23	0.19
PRR 307	0.28	0.23	0.20	0.24
PRR 311	0.27	0.34	0.22	0.24
PRR 314	0.44	0.41	0.43	0.41
PRR 317	0.29	0.27	0.21	0.24
PRR 323	0.19	0.21	0.13	0.26
PRR 326	0.21	0.25	0.17	0.26
PRR 329	0.29	0.27	0.23	0.28
PRR 334	0.29	0.25	0.25	0.22
PRR 337	0.26	0.20	0.20	0.15
PRR 342	0.28	0.29	0.23	0.25
PRR 347	0.27	0.27	0.21	0.22
PRR 348	0.27	0.23	0.21	0.14
PRR 354	0.26	0.21	0.21	0.19
PRR 358	0.25	0.23	0.21	0.22
PRR 363	0.14	0.17	0.17	0.19
PRR 367	0.29	0.23	0.19	0.24
PRR 368	0.23	0.17	0.15	0.20
PRR 372	0.27	0.25	0.22	0.20
PRR 376	0.21	0.19	0.11	0.22
PRR 381	0.31	0.31	0.21	0.29
PRR 386	0.29	0.27	0.21	0.22
PRR 390	0.19	0.21	0.13	0.20
PRR 395	0.21	0.20	0.12	0.16
PRR 396	0.21	0.23	0.15	0.24

ICR, iso-cytoplamsic restorer

Table 4 Correlations between hybrid performance and parental genetic distances under four environments

Correlation	A Line	PL	PF	NT	SF	YPP	PL	PF	NT	SF	YPP
		Delhi (Timely sown condition)					Delhi (Late sown condition)				
MPH	RTN 12A	-0.394*	0.213	0.537**	0.104	-0.056	-0.276	-0.044	0.507*	0.196	0.179
	IR 58025 A	0.075	0.441*	0.103	-0.014	-0.007	0.136	-0.292	-0.226	-0.064	0.143
	IR 79156 A	0.293	-0.115	-0.384	-0.14	0.192	0.128	-0.184	-0.002	-0.015	0.143
	Pusa 6A	0.303	0.35	0.07	-0.075	0.093	-0.088	-0.032	-0.033	0.148	0.008
BPH	RTN 12A	-0.323	0.272	0.427*	0.087	0.027	-0.137	-0.151	0.372	0.098	0.232
	IR 58025 A	0.278	0.339	0.1	-0.083	0.11	0.217	-0.435*	-0.142	-0.031	0.206
	IR 79156 A	0.386*	-0.189	-0.268	-0.193	0.311	0.194	-0.401*	-0.018	-0.005	0.182
	Pusa 6A	0.337	0.265	0.045	-0.047	0.142	-0.064	-0.07	0.131	0.116	0.011
		Pusa, Bihar					*Karnal*				
MPH	RTN 12A	0.274	-0.192	-0.050	0.281	0.037	0.195	0.212	0.037	-0.054	0.615**
	IR 58025A	0.171	0.010	-0.124	-0.078	-0.024	-0.102	0.197	-0.214	0.115	-0.226
	IR 79156 A	0.125	-0.372*	-0.101	0.023	-0.336	0.097	-0.110	-0.064	-0.064	-0.169
	Pusa 6A	-0.189	-0.025	-0.314	-0.105	-0.163	-0.190	0.010	0.056	-0.146	-0.262
BPH	RTN 12A	0.174	-0.238	-0.136	0.161	-0.039	-0.049	0.586**	0.258	0.117	0.003
	IR 58025 A	0.177	-0.100	-0.175	-0.053	-0.020	0.123	-0.118	-0.076	-0.090	-0.247
	IR 79156 A	0.122	-0.411*	-0.246	-0.013	-0.329	-0.073	-0.134	0.131	-0.164	-0.120
	Pusa 6A	-0.151	-0.096	-0.254	-0.025	-0.063	-0.203	-0.145	-0.065	-0.139	-0.050

NT, Number of tillers; PL, panicle length; SF, per cent spikelet fertility; PF, per cent pollen fertility; YPP, yield per plant in grams; MPH, mid parental heterosis; BPH, better parent (iso-cytoplasmic restorer lines) heterosis. *,** significant at $p<0.05$

maximum heterosis was shown in late sown conditions of Delhi (252.60%) with an average value of 82.92%. Thus, in late sown conditions hybrids performed better than the parental lines.

Correlation analysis between GD and heterosis showed that GD was equally correlated with either the average or the control heterosis for each of the six traits. Under normal sown conditions of Delhi, IR 79156A (panicle length, 0.386) and RTN 12A (number of tillers, 0.427) were significantly correlated with GD. Under late sown conditions of Delhi, both IR 58025A and IR 79156A were negative but significantly correlated for pollen fertility percentage (-0.435, -0.401). This demonstrated that the parent's GD used in this experiment only reflected the advantage of the size of the actual output, but not other traits of heterosis, suggesting that the usefulness of marker based GD estimates for predicting yield-related traits.

Due to the simplicity of using DNA molecular markers, marker based estimates of GD has been widely used in predicting heterosis in maize (Lee *et al.* 1989), and rice (Waters *et al*. 2015). Nevertheless, there are studies in which the heterosis prediction using molecular marker based GD has produced inconsistent results (Zhao *et al*. 2008). In the present study also, although a few significant correlations were observed between GD and heterosis for some traits, there were several traits and environmental conditions in which these correlations were insignificant. This demonstrated that the parent's GD based on a set of random SSR markers may not helpful for predicting heterosis for all the traits in question and all environmental situations, which may due to the fact that majority of SSR markers are present in non-coding regions of genome (Krishnan *et al.* 2013). Therefore, use of gene linked or gene based markers for various genes determining yield and yield component traits/need sufficiently larger number of high throughput markers like single nucleotide polymorphisms (SNPs) to determine the parental genotypic differences which would reflect the actual diversity between the parents.

In the present study, GD was significantly correlated with heterosis for number of tillers, pollen fertility percentage and yield per plant underspecific environments and cross combinations. Therefore, it may be prudent to use either functional markers/gene based markers for yield and yield component traitsfor assessment of GD, which may improve the efficiency of prediction of heterosis in rice.

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