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Physiological and molecular basis of water-deficit stress tolerance in F_1 hybrids and their parental lines in rice

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Abstract

Rice hybrids are known to withstand moisture deficit stress better than their parental purelines. To study the effect of water deficit stress in rice hybrids, in terms of productivity related traits and physiological parameters pertaining to drought stress, 22 rice hybrids and their parental lines were evaluated under well irrigated and water deficit conditions. Sixteen F_1 hybrids showed positive heterosis over their respective mid-parental means for yield and spikelet fertility. Pusa 6A and Vandana was the best cross combination for developing high yielding hybrid rice varieties under drought since they had the highest positive standard heterosis and heterobeltiosis for grain yield, spikelet fertility and membrane stability index (MSI). Among the physiological parameters, MSI was found to reflect the drought tolerance ability of a genotype (pureline/hybrid) better. A set of 60 genome-wide SSR markers were used for prediction of heterotic potential of genotypes under well irrigated and water deficit conditions which revealed that genetic distances and spikelet fertility had a positive and significant correlation.

Key words: Grain yield, heterosis, membrane stability index, rice, water deficit

Introduction

Rice (*Oryza sativa* L.), the staple food for half of the world population, is cultivated over 160.9 M ha with 747.5 million tons production (FAO 2014). India is the second largest producer and consumer of rice after china. In India, rice constitutes 22.5% of the gross cropped area yielding 104.4 million tons of head rice/ paddy (commodity profile for rice-March 2015 from http://www.agricoop.nic.in). However, India is supposed to increase its rice production to 156 million tons by 2030 in order to feed its burgeoning population (Goyal and Singh, 2002). Heterosis, also called as hybrid vigor, has contributed tremendously to increased productivity in many crops and is expected to play a pivotal role in enhancing productivity in the future too. In rice, heterosis is being exploited commercially in China, India, Vietnam and the Philippines. Davis and Rutger (1976) and Virmani et al. (1981) extensively reviewed heterosis for various agronomic traits of rice. In many crops, particularly rice, F_1 hybrids are long known to be better at tolerating drought stress than their parental lines (Solomon et al. 2007). Though there are several studies on drought tolerance in rice, as such, there is no systematic investigation on drought tolerance of F_1s and the mechanisms responsible for it.

Drought caused by moisture stress is considered as the single most critical threat to rice production and hence the food security. Considering the large amount of water consumption in rice cultivation and changing climate scenario, drought remains the most important abiotic constraint to rice production (Venuprasad et al. 2007; Berneir et al. 2008). Water stress causes serious damage to rice plant which eventually affects the growth, development and productivity of the plant. To meet the increasing global demand of rice and challenges of abiotic stress, there is a greater need to understand abiotic stress tolerance (Nguyen and Ferrero, 2006; Bouman et al. 2007), especially drought tolerance. A large number of QTLs for various physiological, productivity and root traits

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under drought stress have been reported in rice i.e. root length (Gomez et al. 2010), plant height (Bernier et al. 2007), root dry weight (Zhang et al. 2001), grains per panicle (Babu et al. 2003), relative water content (RWC) (Kanbar et al. 2003), biomass, basal root thickness, osmotic adjustment (Price et al. 2002, Robin et al. 2003) etc. A number of studies have also been conducted in rice for water stress tolerance in different tissues (Wang et al. 2011), in contrasting genotypes (Wang et al. 2007; Lenka et al. 2011) and in mutant lines (Lima et al. 2015). Molecular analysis has suggested that drought-responsive transcription factors (TFs) such as DREB1/CBF, DREB2, AREB/ABF, and NAC TFs function in drought responses and tolerance (Nakashima et al. 2014). Recently, QTL analyses have also revealed novel genes such as *DEEPER ROOTING 1* (*DRO1*) which controls root growth angle in rice (Uga et al. 2013).

Drought stress affects several physiological processes and induces various physiological responses in plants, which help in acclimatizing to such harsh environmental conditions. Thus optimizing these physiological processes becomes a prerequisite for increased productivity under water stress (Serraj et al., 2009). Water deficit alters rice physiology by interfering in process such as relative water content (Lv et al., 2007; Biswas and Choudhuri; 1984; Pirdashti et al., 2009; Cha-um et al., 2010), chlorophyll content (Pirdashti et al. 2009; Cha-um et al. 2010; Sikuku et al., 2012; Ha 2014; Maisura et al., 2014) and membrane stability (Premachandra et al., 1991; Tripathy et al., 2000; Kumar et al., 2014). Increased crop yield and water productivity require the optimization of the physiological processes involved in the critical stages of plant response to soil drying, water-use efficiency, and dehydration-avoidance mechanisms. Elaborate studies considering the physiological responses of rice under drought stress will lead to better understanding of the genetic architecture of various physiological traits of rice under water deficit condition.

Since exploitation of heterosis is a key strategy for increasing productivity of crop plants and F_1 hybrids can better withstand drought stress, an experiment was conducted to know the extent of heterosis for productivity and its related traits as well as physiological traits implicated in drought tolerance, under irrigated and drought stress conditions in a set of 22 fertility restorers comprising of both drought tolerant and susceptible genotypes. An attempt was also made to predict heterosis on the basis of genome wide microsatellite markers.

Materials and methods

The research work was conducted in the net house of National Research Centre on Plant Biotechnology, New Delhi to estimate the extent of heterosis of productivity related traits and physiological parameters for 22 F_1 hybrids and their correlation with parental lines under drought stress. Twenty two fertility restorers and a cytoplasmic male sterile (CMS) line, Pusa 6A were taken for study. The geographical origin and response to drought of the materials used in the investigation are summarized in Table 1. Seventeen of the fertility restorers were drought tolerant and the remaining five were drought sensitive. All the fertility restorers were crossed with a CMS line to generate F_1 hybrids. The seeds of the parents and F_1 hybrids were sown in pots in 3 replications. Drought stress was imposed by withholding water for 15 days at active tillering stage. Three plants per replication were selected to record data on filled grains, unfilled grains and spikelet fertility. Fertility indices were calculated based on filled and unfilled grains per plant. Heterosis was calculated based on its increase or decrease in hybrids against their mid-parent values (Lamkey and Edwards, 1999). Heterobeltiosis was also calculated for yield.

Physiological parameters *viz*., chlorophyll content, RWC and membrane stability index (MSI) were measured under both irrigated and drought stress conditions. RWC of leaf discs was measured according to Barrs (1968). The flag leaves were cut into 2 cm pieces and weighed to record fresh weight (FW). The leaf pieces were then placed in distilled water for 4 h and re-weighed to obtain turgor weight (TW). The leaf pieces were oven dried, weighed to get dried weight (DW). RWC was calculated using the formula RWC=FW-DW/TW-DW*100. MSI was determined according to Leopold et al. (1981). The leaves from control and stressed plants were collected and washed five times with deionized water. Then the samples were chopped into segments and kept in a capped vial with 10 ml deionized water for 24 h at room temperature followed by 20 min autoclave. Electrolytic conductance was measured using a conductivity meter both before autoclaving and after cooling of autoclaved samples. MSI was calculated as the reciprocal of the cell membrane injury after stress, according to the formula, $\text{MSI }\%=[(1-(T_1/T_2))/(1-(C_1/C_2))]\text{x}100$, where T and C refer to the stress and control samples, respectively; the subscripts 1 and 2 refer to the initial and final conductance readings, respectively. Chlorophyll content was measured using SPAD- 502 portable chlorophyll meter which measures the

Table 1. Details of rice genotypes used in the study

Genotypes	Parentage	Ecology	State	Response to drought
CSR 20	CSR 5/Palman 579	Irrigated	Haryana	Susceptible
Vikramarya	RPW 6-13/PTB 2	Irrigated Medium	Andhra Pradesh	Moderately tolerant
Kanak	Jaya/BR 34	Rainfed lowland	Bihar	Tolerant
Kasturi	Basmati 370/CRR 88-17-1-5	Irrigated	Punjab, Haryana, Western U.P. (CVRC)	Sensitive
Pusa 44	IARI-5901-2 x IR-8	Irrigated	Karnataka, Kerla, Punjab (CVRC)	Sensitive
Daya	Kumar/CR 57-49	Irrigated	Odisha	Tolerant
Heera	CR 404-48/CR 289-1208	Rainfed Upland	Odisha	Tolerant
Kalinga-II	Dhunghansali/IR 8	Upland	Odisha	Tolerant
Prasad	IR 747B-26-3/IR 57948	Irrigated	Uttarakhand	Tolerant
Nagina 22	Selection from Rajbhog	Rainfed Upland	Uttarpradesh	Tolerant
Sona Mahsuri	Sona/Mahsuri	Rainfed Shallow	Andhra Pradesh Lowland	Sensitive
Sahbhagi Dhan	IR 55419-04*2/Way Rarem	Rainfed Upland	Jharkhand (CVRC)	Tolerant
Vandana	C 22/Kalakeri	Rainfed Upland	Bihar	Tolerant
Govind	IR 20/IR 24	Rainfed Upland	Uttarpradesh, Uttharakhand (CVRC)	Tolerant
Samleshwari	R 310-37/R 308-6	Rainfed Upland	Chattisgarh	Tolerant
Nilagiri	Suphala/DZ-12	Rainfed upland	Odisha	Moderately tolerant
Rasi	TN1/CO 29	Rainfed Upland	Karnataka, Tamilnadu (CVRC)	Tolerant
Keshava	WGL 28712/IR 36-1996	Irrigated Mid-early	Andhra Pradesh	Moderately tolerant
Karjat-184	TN1/Kolamba 540	irrigated early	Maharastra	Tolerant
ADT-38	IR 1529-680-3-2/IR 4432-52-6-4//IR 7963-30-2	Irrigated	Tamil nadu	Tolerant
Intan	Introduction from Indonesia	Rainfed Shallow Lowland	Karnataka	Tolerant
CSR 30	BR4-10/Basmati 370	Irrigated	Haryana	Sensitive
Pusa 6A	IR 58025A x Pusa 150 after 6 back cross	Irrigated	IARI, New Delhi	Sensitive

greenness or the relative chlorophyll concentration of leaves. The meter makes instantaneous and non-destructive readings on a plant based on the quantification of light intensity absorbed by the tissue sample. All the physiological measurements were done using three biological and three technical replications. The statistical analyses were performed using online tools available at Indian Agricultural Statistics Research Institute website, New Delhi (http://iasri.res.in/analysis/online_analysis.htm) while descriptive statistics was calculated using NCSS Software.

The parental lines were genotyped using 60 genome-wide SSR markers to understand the genetic relationship of the parental lines for each cross combination and their bearing on yield heterosis under well irrigated as well as drought conditions. Leaf samples of each accession were collected and stored at –80°C. DNA was isolated following CTAB procedure (Doyle 1991). PCR amplification for each microsatellite locus was performed according to protocols described by Chen et al. (1997) with slight modification. A total reaction volume of 10 µl containing 20 ng genomic DNA, 2.0 pmol of each primer, 1 µl of 10X buffer (0.1

M Tris pH 8.8, 0.5 M KCl, 15 mM $MgCl_2$, 0.1% gelatine), 200 µM each of dNTPs and 0.3 U of Taq DNA polymerase was used for PCR amplification. The PCR cycling parameters were as follows: 4 min at 94°C; 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 52°C to 60°C (depending on the primer sequence) and 1 min extension at 72°C in each cycle followed by 20 min at 72°C for final extension. The PCR products for four different microsatellite markers, each labelled with a different dye, were multiplexed in a ratio of 1: 1: 2: 4 for FAM: VIC: NED: PET, respectively, to compensate for the differences in the signal intensity of the dyes. One µl of multiplexed sample was mixed with 8.9 µl of Hi-Di formamide and 0.2 µl of an internal size standard ROX500 (Applied Biosystems, Foster City, California, USA), and denatured at 95°C for 5 min. To resolve the amplicons, samples were injected into ABI DNA analyzer, 3730xl (Applied Biosystems, USA) and raw results were analyzed with GENE MAPPER 4.1 software (Applied Biosystems, 2009). Allele binning and allele calling were carried out as described in GENEMAPPER 4.1 manual, (Tiwari et al. 2015). The genetic distance (GD) was calculated according to Nei et al. (1979) and the GD based Neighbor joining tree was constructed using PowerMarker V3.25. Polymorphism information content (PIC) for all the SSRs was also calculated using PowerMarker V3.25. To predict heterosis based on genetic distances, Pearson's correlation coefficients were calculated between heterosis of a hybrid and the genetic distance between its parents considering all the loci.

Results and discussion

The present research work was carried out to determine the heterotic potential of F_1 hybrids in terms of their productivity related traits and physiological parameters under drought. The performance of all the hybrids and their parents was evaluated under well irrigated and drought stress conditions. The descriptive statistics of parents and their hybrids for various traits under study is presented in Table 2. The mean yield of parents under stress was found low (15.13 g/plant) as compared to control (27.44 g/plant). Though the hybrids also exhibited the same trend, they had comparatively lower mean yield (24.04 g/plant) under control but higher mean yield (18.38 g/plant) under stress as compared to the purelines. We observed more than 20% coefficient of variation (CV) for chlorophyll content, filled grains, unfilled grains, total grains and yield in parents and F_1 hybrids under both well irrigated conditions and stress conditions. Parents showed lesser CV under drought stress as compared to irrigated

Table 2. Descriptive statistics of parents and hybrids under well irrigated (C) and water deficit condition (S)

Parents	RWC (%)		MSI (%)		CHL (µg cm^{-2})		FG		UFG		TG		SF (%)		Yield (g)	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Mean	80.59	76.1	74.05	85.64	34.46	34.41	132.1	98.74	30.68	47.63	163.3	146.03	80.82	67.39	27.44	15.13
SD	9.36	12.66	15.57	5.92	7.17	7.48	40.67	27.94	15.48	18.12	43.87	34.85	9.05	10.27	12.95	6.88
Range	60.56-97.55	41.78-90.84	34.11-95.92	71.28-95.34	12.57-42.4	12-45.27	52.99-229.93	49.77-166.64	8.41-87.25	22.26-90.33	93.14-254.01	91.54-238.264	56.52-92.52	35.7-82.52	11.69-66	3.94-29.86
CV	11.61	15.73	21.02	6.91	20.8	21.73	30.78	28.29	50.47	38.05	26.86	23.86	11.2	15.24	47.19	45.47
Hybrids																
Mean	81.47	77.3	79.27	87.76	35.81	33.37	138.1	105.57	43.51	52.87	181.69	158.68	76.11	66.27	24.04	18.38
SD	7.4	6.24	12.57	5.79	4.96	8.07	34.03	35.74	21.8	22.44	35.24	37.25	11.42	13.47	5.47	6
Range	66.11-88.15	66.51-89.53	51-91.34	5.8-96.9	24.97-44.5	12.4-40.54	53.17-202.1	44-177.3	18.43-120.48	26.1-123.65	113.62-265.41	90.8-223.23	30.65-89.43	28.51-82.1	15.21-38.9	8.82-31.29
CV	9.09	7.76	15.86	6.6	13.84	24.19	24.63	33.86	50.11	42.44	19.4	23.48	15.01	20.32	22.75	32.65

RWC: Relative water content, MSI: Membrane stability index, CHL: Chlorophyll content, FG: Filled grains, UFG: Unfilled grains, TG: Total grains, SF: Spikelet fertility conditions for all the productivity related traits other than spikelet fertility. However, in hybrids, other than unfilled grains, all productivity related traits had increased CV under drought stress as compared to well irrigated conditions. All the physiological parameters *viz.*, chlorophyll content, RWC and MSI had low variability in parents as well as in hybrids, the only exception being chlorophyll content in hybrids under well irrigated conditions. The analysis of variance (ANOVA) of parents and their hybrids for the traits under study is presented in Table 3a and b. ANOVA revealed that differences among almost all the traits were significant at 5% (*p* = 0.05) level of probability (Tripathy et al. 2000).

The Pearson's correlation coefficients among different traits observed under normal and drought stress conditions are presented in Table 4. Significant correlations were observed only for 26 out of 136 pairs of comparisons. Five and eight of them were within control and stress, respectively whereas 13 (50%) of the correlations were between control and stress conditions. There were only four negative correlations and all of them involved spikelet fertility and unfilled grains within and between control and drought stress conditions. Among the physiological parameters, only MSI under control showed significant positive correlation with productivity traits such as total grains under control and spikelet fertility under stress. Significant and positive correlations were reported earlier between yield and physiological attributes like proline content, leaf area index, RWC and plant biomass under drought stress condition (Kumar et al. 2014). In our study, though RWC *per se* did not show any significant correlation with productivity traits it did show positive correlation with chlorophyll content under stress. MSI alone showed

Table 3a. Analysis of variance for productivity related and physiological traits in parents

Source	d.f.	Filled grains		Chlorophyll content		MSI		RWC		Unfilled grains		Total grains		Spikelet fertility		Yield	
		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.	
		C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Genotypes	22	4642.53	1792.78	160.74	174.19	737.26	107.42	268.78	500.88	733.56	975.75	5350.16	2769.93	256.92	328.59	521.18	127.09
Replication	2	5.14	2.42	19.79	3.64	2.21	10.59	2.74	0.86	0.52	1.58	9.39	7.71	1.43	1.29	0.36	0.19
Error	46	5.39	2.51	20.08	3.68	2.25	10.54	1.86	0.61	0.51	1.40	9.70	8.40	1.33	1.44	0.30	0.13
CD*		3.81	2.60	7.36	3.15	2.46	5.33	2.24	1.29	1.17	1.94	5.11	4.76	1.89	1.97	0.90	0.59

*5% level of significance. C: Control, S: Stress

Table 3b: Analysis of variance for productivity related and physiological traits in hybrids

Source	d.f.	Filled grains		Chlorophyll content		MSI		RWC		Unfilled grains		Total grains		Spikelet fertility		Yield	
		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.		M.S.	
		C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Genotypes	21	3567.40	4331.13	76.88	650.83	377.41	103.57	144.42	123.39	1491.26	2414.90	3861.99	5254.36	407.07	998.84	93.65	154.48
Replication	2	5.97	7.05	10.23	10.42	1.89	8.74	1.68	0.69	0.51	2.37	10.24	6.22	1.37	1.46	0.17	1.46
Error	44	5.85	6.71	9.61	9.89	1.65	7.78	1.08	0.67	0.54	1.57	10.28	6.31	1.34	1.41	0.18	0.12
CD*		3.99	4.27	5.12	5.19	2.12	4.60	1.72	1.35	1.21	2.07	5.29	4.14	1.91	1.96	0.71	0.58

*5% Significance C: Control, S: Stress

Table 4. Linear correlation coefficients among productivity related and physiological traits under control and drought stress

	Control								Stress							
Traits	FG	UFG	TG	SF	Yield	Chl	MSI	RWC	FG	UFG	TG	SF	Yield	Chl	MSI	RWC
FG_C	1.00															
UFG_C	–0.07	1.00														
TG_C	0.88**	0.42**	1.00													
SF_C	0.49**	–0.87**	0.03	1.00												
Yield_C	0.16	–0.19	0.06	0.21	1.00											
Chl_C	0.09	0.06	0.10	0.00	–0.08	1.00										
MSI_C	0.21	0.26	0.32*	–0.14	–0.06	–0.03	1.00									
RWC_C	0.04	–0.17	–0.04	0.19	–0.15	–0.09	0.06	1.00								
FG_S	0.75**	–0.11	0.62**	0.42**	0.06	0.19	0.17	0.10	1.00							
UFG_S	0.12	0.50**	0.36*	–0.41**	–0.10	–0.23	0.08	–0.04	–0.09	1.00						
TG_S	0.73**	0.18	0.75**	0.14	–0.01	0.04	0.19	0.08	0.83**	0.48**	1.00					
SF_S	0.30*	–0.45**	0.04	0.56**	0.11	0.31*	0.05	0.14	0.64**	–0.80**	0.11	1.00				
Yield_S	0.22	–0.09	0.15	0.19	–0.17	–0.08	0.31	0.28	0.36*	–0.22	0.19	0.38**	1.00			
Chl_S	–0.19	–0.27	–0.30*	0.11	0.07	0.12	0.07	–0.09	–0.14	–0.18	–0.21	0.06	–0.13	1.00		
MSI_S	–0.09	0.05	–0.06	–0.08	–0.23	0.16	0.16	–0.03	0.01	–0.07	–0.03	0.08	0.42*	0.04	1.00	
RWC_S	–0.05	–0.10	–0.09	0.07	–0.01	0.01	–0.03	–0.09	–0.03	0.11	0.03	–0.09	–0.07	0.47*	0.11	1.00

FG: Filled grains, UFG: Unfilled grains, TG: Total grains, SF: Spikelet fertility, Chl: Chlorophyll content, MSI: Membrane stability index, RWC: Relative water content; **1% level of significance; *5% level of significance

positive and significant correlation with yield under stress in our data. This suggested that MSI could be a better physiological parameter of selection to identify drought tolerant genotypes. Tripathy et al. (2000) also found significant correlation between MSI and yield under drought stress in rice. Chlorophyll content under stress also showed positive correlation with spikelet fertility under stress. Thus, most of the correlations within physiological parameters and, between physiological parameters and productivity traits were observed only under stress.

The heterotic performance of 22 F_1 hybrids for various productivity related and physiological traits are presented in Table 5. All the combinations showed different degree of heterosis for individual traits (Yanal et al. 2013). Heterosis for yield *per se* ranged from –21.5 to 6.6 g/plant under control and –12.9 to 12.1 g/plant under stress. The highest mid parental heterosis for yield was observed for hybrids of Nagina 22 and Vandana. Two more hybrids, from Govind and Samleswari had positive heterosis for yield and its related traits and they also had high membrane stability (~87%). These two hybrids, besides that of Sahbhagidhan and Sona Mahsuri also had positive heterosis for spikelet fertility under stress. Overall, heterosis for spikelet fertility ranged from –56.9 to 17% under control and –38.7 to 24.2% under stress. Nearly 70% of the hybrids (16 out of 22) showed better heterosis for spikelet fertility under stress while it was found negative in most of the hybrids under control conditions. Vandana had the highest magnitude of heterosis for both yield and spikelet fertility. Besides, Govind, Samleswari, Nilagiri, Keshav and Intan showed better heterotic performance for all productivity related traits while Rasi, Karjat-184 and ADT-38 showed negative heterosis for most of the yield and its related traits. Rasi is a well known drought tolerant rice genotype which is also evident from our data under stress conditions but its yield reduced significantly in hybrid under drought which indicates that the genetic background of Pusa 6A adversely affected the drought tolerance mechanism of Rasi genotype.

Heterobeltiosis was carried out for yield to compare the hybrid over better parent (Table 6). Heterobeltiosis for yield ranged from –34.56 to 11.71 g/plant under control and -18.08 to 19.09 g/plant under stress. Under well irrigated condition, 12 out of 22 F_1 hybrids showed significantly better yield compared to

Table 5. Mid parent heterosis for productivity related traits

F_1 hybrids with genotypes	Yield (g)		SF (%)		FG		UFG		TG	
	C	S	C	S	C	S	C	S	C	S
CSR 20	–7.7	–9.4	–11.2	0.6	–7.8	–0.5	19.1*	–3.8	11.7*	–5.2
Vikramarya	0.7	5.3*	–4.0	10.5*	14.2*	13.5*	7.7*	–17.7	20.1*	–3.4
Kanak	–4.5	–3.2	–15.0	3.7*	40.8*	14.8*	47.0*	1.2	87.3*	17.9*
Kasturi	6.0*	–0.2	–12.1	–2.1	15.6*	10.0*	30.3*	10.7*	49.8*	23.0*
Pusa 44	–21.5	7.5*	–1.3	–4.6	16.0*	–5.2	3.7*	9.4*	17.8*	7.0*
Daya	–9.1	–1.3	–1.6	15.0*	11.5*	20.4*	6.3*	–24.3	19.0*	–3.5
Heera	2.3*	6.0*	3.0*	–7.4	50.0*	–21.0	16.8*	–6.4	65.9*	–26.9
Kalinga II	0.2	4.1*	–9.2	4.7*	16.1*	12.5*	25.6*	–3.1	41.2*	12.5*
Prasad	0.1	–1.3	3.6*	11.4*	14.6*	26.5*	–2.6	–14.7	12.0*	13.1*
N 22	6.6*	10.6*	–9.0	7.1*	–4.4	24.0*	19.7*	–6.8	9.7*	18.2*
Sona mahsuri	–4.0	1.7*	1.8	17.2*	8.2*	16.5*	1.9*	–34.2	7.7*	–15.7
Sahbhagidhan	5.8*	9.7*	–14.8	24.2*	–11.1	27.7*	31.8*	–42.3	21.8*	–13.4
Vandana	4.3*	12.1*	3.0*	16.3*	–22.9	–8.6	–11.8	–33.2	–38.2	–41.2
Govind	0.0	3.7*	2.2*	18.5*	38.0*	60.6*	8.1*	–17.0	44.7*	42.6*
Samleshwari	–3.6	7.0*	–9.6	22.7*	46.1*	95.9*	36.7*	–16.0	79.1*	83.8*
Nilagiri	0.7	5.7*	–6.1	10.7*	34.9*	73.0*	22.5*	10.3*	57.7*	81.1*
Rasi	–10.9	–11.5	–56.9	–38.7	–64.5	–38.6	103.1*	75.3*	37.5*	40.0*
Keshav	–19.8	–12.9	2.6*	0.2	35.4*	50.3*	–2.0	25.9*	32.8*	75.9*
Karjat 184	–3.8	0.6*	–10.6	2.2*	–49.9	–36.3	6.2*	–24.4	–44.1	–60.0
ADT 38	–15.8	4.2*	–12.5	17.0*	–24.5	14.2*	17.9*	–28.6	–8.4	–15.5
Intan	3.4*	5.4*	17.0*	16.9*	39.4*	61.9*	–36.6	–11.0	1.1	54.6*
CSR 30	–9.8	–6.5	–8.3	3.0*	15.7*	9.1*	15.9*	–6.2	31.6	5.4*

FG: Filled grains, UFG: Unfilled grains, TG: Total grains, SF: Spikelet fertility, C: Control, S: Stress; * Significant based on CD values of hybrids

parents. In absolute terms, six out of these 12 hybrids had heterosis to the tune of 16%. Under stress condition, around 16 F_1 hybrids showed high yield compared to their respective parents. Hybrids which performed better under normal conditions, also performed better under drought stress. Besides these 16 hybrids which performed better under well irrigated as well as drought stress conditions, four more hybrids of Pusa 6A with Pusa 44, ADT 38, Sona Mahsuri and Vikramarya showed better performance under drought stress condition (Table 6). Thus, our experimental results validated that more often than not hybrids performed better under drought stress (Carnahan et al. 1972; Mohanty and Mohapatra, 1973; Saini and Kumar 1973; Mallick et al. 1978; Virmani et al. 1982; Luat et al. 1985; Moses and Abebe, 2014; Peng and Virmani, 1994; Xiuxiu et al. 2014). Based on heterobeltiosis, Vandana and Pusa 6A are the best specific combiners for yield under drought stress and can be tested at field studies for further validation. Cumulatively, based on the results of both heterosis and heterobeltiosis, the hybrids of Samleswari and Intan also consistently performed better.

To evaluate the genetic differences between the CMS line and the 22 fertility restorers used in the study, they were genotyped using 60 genome-wide microsatellite markers evenly distributed on 12 rice chromosomes through fluorescent dye labelled primers automated fragment analyzer. Four major clusters were observed among the 23 genotypes. The CMS parent, Pusa 6A was with Intan, Pusa 44 and Nilagiri in the fourth cluster. Vandana and Nagina 22 were placed in the second cluster and they showed better heterosis with Pusa 6A. However, overall, the SSR diversity based distance between the parental combinations, did not have any bearing on the hybrid vigour of respective F_1s. PIC values and genetic distance estimates revealed that some of the parents were genetically nearly identical (Rasi and Samleswari with distance estimate of 0) whereas some were quite unrelated (CSR30 and Keshav with maximum distance of 0.84). Still, Rasi and Sameshwari had heterosis in opposite directions for yield and many of its related traits (Table 5).

Table 6. Heterobeltiosis based on yield

F_1 hybrids with genotypes	Control	Drought stress
CSR 20	–7.92	–9.46
Vikramarya	0.26	4.88*
Kanak	–3.93	–3.08
Kasturi	11.06*	2.74*
Pusa 44	–34.56	14.84*
Daya	–10.73	–0.61
Heera	9.31*	9.22*
Kalinga-II	3.28*	4.24*
Prasad	5.12*	1.30*
Nagina 22	8.12*	11.86*
Sona Mahsuri	–4.60	3.59*
Sahbhagi Dhan	1.06*	7.58*
Vandana	11.49*	19.09*
Govind	5.11*	7.69*
Samleshwari	0.88*	13.95*
Nilagiri	8.48*	10.54*
Rasi	–11.98	–12.43
Keshav	–26.46	–18.08
Karjat-184	1.50*	8.34*
ADT-38	–34.56	6.66*
Intan	11.71*	10.73*
CSR 30	–8.90	–3.35
Pusa 6A	–5.98	–3.12

Table 7. Coefficients of correlation between parental genetic distance and heterosis

Trait	Control	Drought stress
Yield	0.06	–0.06
Filled grain	–0.15	0.04
Unfilled grain	0.18	–0.41
Total grains	–0.04	0.07
Spikelet fertility	–0.17	0.43*
Chlorophyll content	–0.02	0.04
MSI	–0.21	0.18
RWC	0.02	–0.10

*$p < 0.10$

To increase the hybrid breeding efficiency, DNA markers have been used to investigate the parental genetic distance and its relationship with heterosis (Caruso et al. 2010). DNA markers have successfully been used in several studies for predicting heterosis and hybrid performance (Zha et al. 2008; Jaikishan et al. 2010; He et al. 2002). In this study, we employed microsatellites for predicting heterosis of rice hybrids under well irrigated and drought stress through correlation between microsatellite-based genetic distances of parents and their respective hybrid's heterosis (Table 7). The correlation between parental genetic distance and heterosis was investigated by analyzing the performance of all the F_1 Hybrids. The results showed significant correlation between genetic distance and spikelet fertility ($r = 0.43$; $p < 0.10$). It is worthwhile to note here that spikelet fertility also showed positive heterosis in 16 out of 22 hybrids. For other pairs, the correlation was found very low or with negative *r* values. Non-significant relationship between SSR markers based genetic distance and heterosis could be because SSRs represented a genome-wide diversity, whereas heterozygous loci for each trait could be localized to a specific region (Jaikishan et al. 2010; He et al. 2002). Heterosis prediction using functional markers might be able to provide better results rather than random genome-wide markers as carried out in our study (Jaikishan et al. 2010). In the present study, molecular markers were not suitable for prediction of hybrid performance for most of the traits, other than spikelet fertility. The information generated from this study will be useful for future rice stress breeding programmes.

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