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Parental molecular diversity and its concurrence to heterosis in bread wheat (*Triticum aestivum*)

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ABSTRACT

A half diallel set of 10 parents, 45 F_1s and 45 F_2s was analyzed for grain yield and its components and parental entries were also assessed with RAPD marker for the concurrence of genetic diversity analysis with heterosis and combining ability. Out of 45 crosses, thirteen-thirty one crosses manifested heterotic response for different characters in desired direction. An overall appraisal of GCA effects identified parents DBW 16 and UP 2338 as good general combiners for grain yield and its contributing traits and crosses DBW 16 × UP 2338, DBW 17 × UP 2338 and PBW 373 × Raj 4083, as having high significant positive SCA effects for grain yield as well as for its components. RAPD analysis discemed 352 fragments of which 95 scorable alleles were obtained with 16 primers with an average of 5.93 alleles/primer.

Key words: GCA effects, Heterosis, Inbreeding depression, SCA effects, RAPD and bread wheat

To breed the new improved genotypes, one of the most important factors in determining feasibility of hybrid wheat (*Triticum aestivum* emend Fiori & Paol.) is the nature and amount of heterosis and its exploitation. The study of heterosis helps the plant breeder in eliminating the less productive crosses in early generations. In addition to the understanding of heterosis in wheat, it is also necessary to find out gene actions involved in the expression of quantitative and quality traits of economic importance. The combining ability determined through diallel analysis is useful to assess the nicking ability of the parents and at the same time it elucidates the nature and magnitude of different types of gene actions involved. This, in turn, helps in determining the most appropriate breeding methodology to be adopted to achieve maximum genetic improvement in our breeding programmes. Further, based on DNA profiles of characterized objects it is possible to determine their genetic similarity. The knowledge of genetic diversity on molecular level may be helpful with choosing the appropriate parents for breeding hybrids (Krystkowiak *et al.* 2009, Myskow *et al*. 2010).

MATERIALS AND METHODS

The present investigations were conducted at Instructional Farm of Rajasthan College of Agriculture, Udaipur, India (579.50 m above mean sea level latitude of 24°–35' N and longitude of 70°–42'E) during winter (*rabi*) season of 2008–09. Ten diverse wheat genotypes namely, Raj 1482, PBW 502, PBW 343 , PBW 373 , DBW 16, DBW 17, HD 2687, UP 2338, Raj 4083 and Raj 4037 were selected as parents on the basis of their origin, adaptability, diversity, yield potential and heat tolerance characters. Crosses were attempted during *rabi*, 2007–08 in diallel fashion (excluding reciprocals). Further the F_1s were multiplied during off-season at Indian Agricultural Research Institute, Regional Research Station, Wellington, Tamil Nadu so as to obtain F_2 generation. Final experimental trial comprising 10 parents along with 45 F_1s and 45 F_2s was evaluated during *rabi,* 2008–09 in randomized block design with two replications at Udaipur. Parents, F_1s were grown in single row while F_2s in three rows. The observations on seven traits, viz days to heading, plant height, number of effective tillers/plant, flag leaf area, 1000-grain weight, biomass/plant and grain yield/ plant were recorded on ten randomly selected competitive plants in parents and F_1s while 30 in F_2s/treatment/replication. Heterosis, heterobeltiosis and inbreeding depression were calculated as per standard procedures. The combining ability analysis was carried out according to method 2, Model-I of Griffing (1956). All the parents were also studied for genetic diversity using RAPD for the concurrence between RAPD and morphological markers along with their known pedigree. Healthy seeds with identical dimensions were selected by visual observation, washed then surface sterilized by 0.1% $HgCl_2$ for 2 min. and germinated on filter papers in dark at room temperature. The leaves were harvested after 15 days

Based on Ph D work

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and DNA was isolated with CTAB method. DNA was purified by using RNAse, Protinase and sequenced washing with ethanol and compared with λ Hind III bacteriophage DNA through running on 0.8% agarose gel. A set of 16 decamer primers were screened. PCR was performed in 25 µl reaction tube with 5 ng genomic DNA, 0.75 µl of dNTPs, 1.00 µl of each primer and 0.5 unit of *Taq* polymerase, maintained in 2.5mM $mgcl_2$. The thermal profile of PCR was 45 cycles at 94° C, 60 s; 35° C, 60 s and 72° C, 120 s. before the first cycle the temperature of 94° C was maintained for 4 min and after the last cycle 72° C was maintained for 7 min. On completion of the reaction, submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.5% agarose gel. Photographs from ethidium bromide containing gel were used to score the data manually and independently. Presence of amplified product were scored as 1 and its absence as 0 for all genotypes and primer combinations. These data matrices were analyzed using NTSYS-PC. The Jaccard's similarity coefficient was used to construct dendrogram based on UPGMA.

RESULT AND DISCUSSION

The analysis of variance for experimental design was performed for seven characters. It revealed significant differences for all the characters indicating presence of adequate genetic variation among the genotypes. Further partitioning of mean squares due to F_1s and F_2s were significant for all the characters, except 1000-grain weight and harvest index in both generations. It revealed that adequate amount of variation was present in F_1s and F_2s. Mean squares due to parent v/s hybrid component were also significant for all the characters which depicted presence of heterosis for all the characters.

RAPD analysis

Primer OPR-15 gave 16 polymorphic alleles in the range of ~ 600bp to ~ 2800 bp with 100% polymorphism while, OPS-06 had given four scorable polymorphic alleles in the range of ~ 700bp to ~ 900 bp including one monomorphic allele (~ 800 bp). The highest numbers of scorable alleles were found in primer OPR-15 which gave 16 scorable alleles while, the lowest numbers of alleles three were obtained with primers OPR-18, OPS-04 and OPS-05. Looking to the investigation 352 fragments were amplified in all genotypes. Ninty-five scorable alleles were obtained with 16 primers with an average of 5.93 alleles/primer. Seventy six bands were found to be polymorphic and the level of polymorphism was 80.00%. The average number of polymorphic bands found to be 4.75/primer. Primers OPR-15, OPR-18, OPS-04, OPS-05 and OPS-06 were all informative and showed 100% polymorphism.

The Jaccard'ssimilarity coefficient displayed in the range of 0.17 to 0.62. The dendrogram (Fig 1) clearly indicated four main clusters. The cluster I included Raj 1482, PBW 502, DBW 17, PBW 373 and UP 2338. The cluster I has two sub-clusters in first sub-cluster Raj1482, PBW 502 and DBW 17 were grouped whereas, in second sub-cluster PBW 373 and UP 2338 were included with 0.46 similarity coefficient. DBW 16 and Raj 4037 were grouped in II cluster and dendrogram showing similarity coefficient 0.51. The third major cluster included HD 2687 and Raj 4083 with 0.38 similarity co-efficient. While the genotype PBW 343 was grouped single in separate fourth cluster, however it is having common pedigree with PBW 373(ND/BG1944//KAL//BB/3/BACO'S'/4/BAA//5'S'). The formation of separate cluster indicated the consequence of segregation at different loci.

Concurrence of RAPD study with heterosis, inbreeding depression and combining ability

The results revealed significant positive as well as negative heterosis and heterobeltiosis in many crosses for different characters studied (Table 1). Heterosis for days to heading ranged from –24.34 (DBW 16 × Raj 4083) to 2.91 (PBW 502 × PBW 343). It was significant in twenty-two crosses, out of which all crosses depicted negative heterosis. Raj 1482 × Raj 4037 exhibited best cross *per se*, significant positive heterosis (–12.60), heterobeltiosis (–11.30) and SCA effects (–5.32) in F_1s and both the parent were good general combiners for plant height in F_1 and Raj 4037 was also found with high GCA effects in F_2 generation (Tables 2,3). However, Raj 1482 and Raj 4037 were released from same source but having different pedigree as Napo-Tobari S/8156/Cal-Db

Table 1 Mean (%) and range of heterosis, heterobeltiosis and inbreeding depression for seven characters in bread wheat

Character	MP		BP		ID	
	Mean	Range	Mean	Range	Mean	Range
Days to heading	8.48	–24.34–2.91	5.64	–19.58–(–)0.65	7.72	–28.57–7.50
Plant height (cm)	4.99	–14.80–9.29	2.93	–12.40–(–)0.58	5.17	–17.33–14.09
Number of effective tillers/plant	35.63	–29.44–158.67	26.42	1.15–137.26	18.31	–80.72–48.51
Flag leaf area (cm^2)	28.62	–51.98–53.48	5.12	6.60–39.63	44.11	–150.37–65.95
1000 grain weight (g)	6.95	–0.61–19.11	4.51	0.28–17.06	4.61	–6.55–15.68
Biomass/plant (g)	23.96	–46.18–87.22	12.57	0.64–65.22	13.95	–16.92–48.04
Grain yield/plant (g)	29.16	–40.99–115.65	18.08	3.11–93.19	16.79	–10.88–49.27

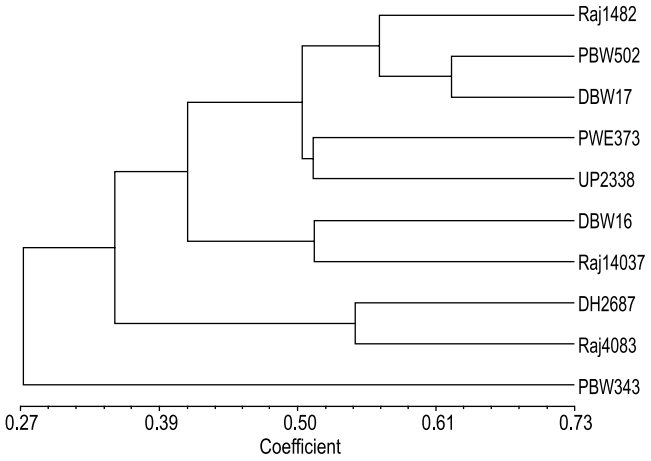

Fig 1 Dendrogram generated using UPGMA cluster analysis based on Jaccard's similarity coefficient

and DL788-2/Raj3717, respectively. Parental genetic distance was also found as 54% and parent were grouped in I and III clusters in RAPD analysis. Only three crosses, viz PBW 373 × Raj 4083, PBW 343 × Raj 4037 and DBW 17 × UP 2338 exhibited significant positive SCA effects in F_1 generation for 1000-grain weight. Parent of the cross PBW 373 × Raj 4083 were 66% genetically dissimilar and were grouped in II and IV clusters respectively in RAPD study.

A perusal of data indicated that heterobeltiosis for grain yield was depicted in fourteen crosses, which ranged from 3.11 (PBW 343 × DBW 17) to 93.19 (DBW 17 × UP 2338). The highest positive heterobeltiosis was noticed by the hybrid DBW 17 × UP 2338 (93.19) followed by Raj 1482 × DBW 17 (68.63) and HD 2687 × UP 2338 (66.98). Results are in agreement with Hassan *et al*. (2006), Saini *et al*. (2006), Ulukan (2007) and Xinnian *et al*. (2007). Based on heterotic studies, the best direct yield contributing character was effective tillers per plant followed by biomass/plant. The results are in conformity with the findings of Bhatt *et al*. (2006) and Vanpariya *et al*. (2006). Crosses, viz PBW 373 × DBW 17, DBW 16 × UP 2338 and DBW 17 × UP 2338 exhibited low magnitude of inbreeding depression for grain yield/plant. The high values for heterotic effects indicated that the parents used for the study appeared to be genetically diverse. Considerable high heterosis in certain hybrids and low in other revealed that nature of gene action varied with the genetic architecture of the parents which might help in identifying superior cross combination. It is well established that there could be no separate gene system for yield *per se* as yield was an end product of the multiplicative interaction between its various components. Thus heterosis for grain yield could be determined by finding the effect of heterosis for individual yield components.

Since SCA effects were related to GCA effects of their parents, performance of crosses on the basis of GCA was more efficient than that of SCA. Therefore, more stress should be laid on GCA effects rather than SCA effects. In the

Table 2 Estimation of GCA effects for days to heading, plant height, number of effective tillers/plant, flag leaf area, 1000–grain weight, biomass/plant, grain yield/plant in bread wheat

Genotype	Days to heading		Plant height (cm)		Number of effective tillers/plant		Flag leaf area (cm^2)		1000–grain weight (g)		Biomass/plant (g)		Grain yield/ plant (g)	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Raj 1482	–0.24	–0.20	–2.56**	–0.33	–0.44	–0.16	–6.82**	2.18**	–0.65	–0.71*	–2.79**	–4.49**	–1.45**	–2.05**
PBW 502	1.55*	1.55*	–1.10	0.72	–0.25	–0.02	–1.27*	2.58**	0.40	0.07	–2.18**	–1.51**	–1.19**	–0.82**
PBW 343	3.34**	0.18	1.22	1.72**	–0.65**	–0.18	–2.96**	–0.94*	0.36	–0.20	–3.20**	–2.74**	–1.47**	–1.36**
PBW 373	3.47**	1.68**	0.76	1.24	–1.25**	–0.56**	1.23*	–2.01**	–0.15	–0.15	1.90**	2.02**	1.06**	1.07**
DBW 16	3.97**	0.63	2.57**	0.16	0.65**	–0.31	0.69	0.50	0.67	1.07**	1.68**	1.25*	0.74*	0.67**
DBW 17	2.22**	1.63**	0.07	–0.88	0.38	–0.31	3.55**	–2.58**	0.17	0.03	2.84**	2.60**	1.52**	1.42**
HD 2687	–1.70**	–2.41**	–0.05	0.45	0.21	0.62**	0.90	–2.52**	0.57	0.68*	0.93	0.74	0.54	0.28
UP 2338	–0.07	0.80	2.35**	0.07	–0.18	0.11	4.14**	2.92**	–0.09	0.02	3.76**	3.37**	1.75**	1.36**
Raj 4083	–6.41**	–2.45**	–1.23	–1.68*	0.37	–0.35	0.38	–0.18	–0.77	–0.90**	–2.24**	–1.60**	–1.11**	–0.73**
Raj 4037	–6.12**	–1.41*	–2.03**	–1.46*	1.16**	1.17**	0.17	0.06	–0.50	0.09	–0.70	0.36	–0.39	0.16
(SE) g_i	0.64	0.62	0.53	0.75	0.34	0.35	0.57	0.40	0.37	0.59	0.31	0.33	0.62	0.33

* Significant at 5 per cent level, **Significant at 1 per cent level

Table 3 Estimation of SCA effects for days to heading, days to maturity, plant height, number of effective tillers/plant, flag leaf area, 1000-grain weight, biomass/plant, grain yield/plant in F_1 and F_2 generations in bread wheat

Crosses	Days to heading		Plant height (cm)		Number of effective tillers/plant		Flag leaf area (cm^2)		1000–grain weight (g)		Biomass/plant (g)		Grain yield/plant (g)	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P1 × P2	–9.16**	–5.20*	0.02	–0.96	3.56**	3.58**	–2.90	12.75**	1.25	1.79	–0.81	–3.87*	–0.79	–1.69*
P1 × P3	–0.95	–4.83*	–3.00	–2.06	0.07	–1.17	–2.58	0.78	1.60	–1.36	1.69	–0.84	0.81	–0.20
P1 × P4	–1.58	0.17	–4.37	–0.57	1.25	0.85	–7.26**	6.31**	1.29	3.25**	–2.60	–5.95**	–0.95	–2.78**
P1 × P5	0.42	1.21	2.47	1.77	–1.10	–0.15	–8.94**	–14.52**	2.16	–1.73	–4.57*	–6.51**	–1.85	–2.57**
P1 × P6	–0.83	–3.79	–0.61	–1.56	–2.55**	–0.53	4.57*	–13.67**	0.88	3.22**	4.55*	–1.13	2.25*	–0.84
P1 × P7	0.09	0.75	–2.04	–2.72	1.55*	1.24	–0.50	–0.43	0.46	–1.26	1.16	1.25	0.64	0.93
P1 × P8	–0.04	–0.95	2.65	5.64*	1.15	0.37	–8.17**	13.99**	–2.20	0.23	–0.36	–3.86*	–0.26	–1.41
P1 × P9	2.80	2.80	–3.67	1.37	–1.08	–0.67	–0.31	21.59**	–0.57	0.54	1.32	9.38**	0.91	4.01**
P1 × P10	0.00	4.75*	–5.32*	–3.43	–0.21	–1.66*	0.25	–15.19**	1.22	–0.80	3.49	2.61	2.26*	1.84*
P2 × P3	4.75*	4.92*	9.43**	–1.21	–1.24	4.05**	–5.62**	–1.58	–1.15	0.03	–1.86	–2.09	–0.32	–1.44
P2 × P4	4.13	2.92	2.95	3.76	0.96	0.13	–7.44**	–11.20**	0.05	–1.80	–3.69	–3.49*	–1.80	–1.58
P2 × P5	2.13	–7.54**	–3.13	0.85	2.43**	–1.33*	–7.85**	0.92	–0.76	0.18	–5.70**	–5.24**	–2.60*	–2.00*
P2 × P6	–2.62	1.96	–6.65**	0.88	1.77*	–0.87	1.34	–13.39**	0.98	–3.86**	5.11**	6.33**	3.28**	3.51**
P2 × P7	–2.20	–3.50	–8.43**	0.72	–0.88	–1.14	–9.98**	7.31**	0.22	0.46	–2.90	–1.25	–1.39	–0.28
P2 × P8	0.67	–6.70**	–0.89	–1.24	–2.31**	–0.13	1.56	–6.56**	2.19	–0.65	2.14	–1.86	0.31	–0.64
P2 × P9	–4.00	4.05*	0.69	0.04	1.29	–0.08	1.32	–2.47	–0.93	1.10	0.40	0.15	0.85	–0.51
P2 ×P10	–2.79	4.00	–0.88	–0.01	–3.38**	–2.98**	3.92*	5.42**	–0.13	1.16	–1.43	–0.70	–0.84	–0.58
P3 × P4	1.84	2.80	0.06	2.78	0.29	–0.89	2.24	20.88**	1.52	0.76	0.72	–0.32	0.16	0.05
P3 × P5	2.84	1.34	–3.90	–3.70	2.98**	1.13	–1.01	–4.41**	–1.40	–0.72	0.80	–1.68	0.37	–0.52
P3 × P6	1.09	–0.16	–1.20	–2.07	–0.77	–0.67	–10.82**	3.12*	–0.05	0.70	–4.67*	–5.05**	–2.31*	–2.20*
P3 × P7	–1.50	–1.12	–1.85	–0.49	1.35	0.04	0.33	–10.66**	1.49	–0.11	4.71*	5.47**	2.69**	2.26**
P3 × P8	0.88	1.17	–5.68*	–0.09	–1.85*	–0.93	–7.21**	–8.66**	–0.45	1.63	–4.29*	–1.74	–1.88	–0.17
P3 × P9	–7.29**	–4.08*	–8.18**	–2.64	–0.77	–2.23**	–10.52**	–14.04**	–0.41	–2.27*	–9.27**	–9.61**	–4.11**	–4.54**
P3 × P10	–2.58	–9.62**	1.97	1.55	–0.23	0.50	13.39**	2.30	3.08*	1.33	6.70**	6.25**	3.87**	3.11**
P4 × P5	2.21	1.34	4.30	4.76*	–0.65	0.12	–6.82**	5.36**	–2.02	1.02	–7.41**	–5.14**	–3.33**	–2.49**
P4 × P6	2.46	0.84	–0.87	0.39	–2.40**	–0.72	0.45	–7.85**	1.76	2.84*	4.88*	2.26	1.94	0.77
P4 × P7	–1.12	–1.12	1.73	0.70	0.59	0.06	16.02**	–10.63**	2.27	1.59	9.04**	9.03**	4.99**	5.00**
P4 × P8	2.75	–1.33	–0.60	0.16	0.85	0.37	7.27**	–11.12**	1.44	–0.69	4.30*	5.59**	2.39*	2.38**
P4 × P9	–7.41**	–5.58**	2.36	–3.86	–0.78	0.40	13.95**	–3.74**	3.94**	–0.39	10.37**	8.04**	5.02**	4.29**
P4 × P10	–4.70*	–4.62*	–2.34	–0.54	0.03	0.67	–15.01**	3.90**	–2.28	–0.59	–6.43**	–6.35**	–2.67*	–3.21**
P5 × P6	3.96	3.38	–3.02	–0.83	1.52	3.68**	–2.36	9.72**	–0.51	1.09	–0.33	1.41	–0.13	0.67
P5 × P7	–2.12	–2.08	7.48**	–4.92*	–0.89	–0.94	–4.44*	–8.97**	–1.14	–2.24*	–5.23**	–2.47	–1.95	–1.84*
P5 × P8	2.75	–0.29	1.59	–2.62	1.97*	–0.10	14.93**	4.65**	2.43	1.31	12.52**	4.80**	5.84**	2.81**
P5 × P9	–9.91**	–6.54**	–3.88	–1.16	0.12	–0.12	–1.40	–0.80	0.77	0.99	1.34	1.25	0.42	0.13
P5 × P10	–7.70**	–5.58**	1.50	2.06	1.24	–0.40	10.61**	1.45	2.03	1.26	10.47**	7.76**	4.96**	3.92**

Contd.

Table 3 *Concluded*

Crosses	Days to heading		Plant height (cm)		Number of effective tillers/plant		Flag leaf area (cm^2)		1000–grain weight (g)		Biomass/plant (g)		Grain yield/ plant (g)	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P6 × P7	–2.87	1.42	1.08	4.78*	1.53	0.36	1.42	–3.02*	–0.78	0.44	0.57	–4.32*	0.19	–1.67*
P6 × P8	–2.00	1.21	3.60	–0.94	1.38	–0.08	4.71*	15.22**	2.70*	–0.95	10.12**	10.56**	5.75**	4.82**
P6 × P9	–2.66	–7.04**	4.23	–5.92**	5.48**	1.20	9.30**	4.78**	1.50	0.37	4.39*	6.38**	2.35*	3.86**
P6 × P10	–3.95	–3.58	2.01	2.48	2.05*	0.99	12.43**	3.08*	–0.23	–0.14	0.75	1.96	0.34	0.96
P7 × P8	–0.58	1.25	4.66	1.44	1.33	1.70*	–1.82	7.62**	1.28	3.35**	6.53**	8.77**	3.78**	3.37**
P7 × P9	–1.75	–7.50**	1.18	2.97	–0.84	–0.23	–0.39	–12.25**	–1.39	–0.66	–0.68	–10.37**	–0.45	–4.63**
P7 × P10	1.96	2.96	–5.58*	0.19	–0.91	1.49*	–14.55**	4.91**	0.34	–0.39	–6.23**	–5.91**	–3.21**	–2.27**
P8 × P9	–2.87	5.30*	–1.70	0.83	0.73	2.16**	–6.42**	–4.11**	0.55	0.41	–6.85**	–6.64**	–3.19**	–2.89**
P8 × P10	–4.16	5.25*	–0.41	–6.66**	1.09	0.27	–5.41**	–15.03**	–2.08	–0.32	–7.63**	–6.69**	–2.85**	–3.31**
P9 × P10	4.17	9.50**	2.16	2.17	1.77*	0.75	–10.36**	5.44**	–0.40	0.43	–2.15	–3.14	–1.28	–1.06
(SE) s_{ij}	2.14	2.01	2.32	2.13	0.77	0.65	1.77	1.25	1.34	1.10	1.91	1.71	1.01	0.83

Here P1- Raj 1482, P2-PBW 502, P3- PBW 343, P4- PBW 373, P5- DBW 16, P6- DBW 17, P7- HD 2687, P8- UP 2338, P9- Raj 4083 and P10- Raj 4037

* Significant at 5 per cent level; **Significant at 1 per cent level

present study, an overall appraisal of GCA effects revealed that DBW 16, DBW 17, UP 2338 and Raj 4083 were good combiner for the majority of characters. High GCA effects are related to additive gene effects or additive × additive interaction effects which, represent the fixable genetic component of variation. Hence, these parents could be efficiently used for exploiting grain yield. In contrast to GCA effects, SCA effect represent dominant and epistatic component of variation which are non-fixable and do not contribute tangibly to the improvement of self-pollinated crops, except where commercial exploitation of heterosis is feasible. In the present investigation, estimates of SCA effects indicated that no cross combination was consistently good for all the characters studied. The hybrid DBW 16 × UP 2338 depicted highest SCA effect followed by DBW 17 × UP 2338 and PBW 373 × Raj 4083 in both F_1 and F_2 generation for grain yield. These crosses involved all the three types of combinations with regards to their significant GCA effects and *per se* performance, viz high × high, high × average and average x low for grain yield and its components. The parents of promising crosses, viz DBW 16 × UP 2338 and DBW 17 × UP 2338 were also 82 and 63% genetically dissimilar, respectively for biomass and grain yield. The manifestation of heterosis and SCA effects for effective tillers/plant, flag leaf area and biomass/plant were responsible for increased grain yield in the crosses DBW 16 × UP 2338 and DBW 17 × UP 2338 these crosses will be gainfully utilized in future breeding programmes.

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