



Hybridization of potential germplasm to create genetic variation for identification of superior cross combinations in bread wheat (*Triticum aestivum*)

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ABSTRACT

A systematic hybridization was made for inducing genetic variation to produce high yielding genotypes with a high degree of heterosis and combining ability. The present study was carried out at during winter (*rabi*) season 2020–21 and 2021–22 at Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh to identify superior genotypes with strong general combining ability (GCA) and crosses with the potential to significantly enhance wheat (*Triticum aestivum* L.) yields in the future. The experiment was conducted with 10 parents to produce 45 hybrids using half diallele mating design, while evaluation of the same 10 parents and 45 hybrids using randomized complete block design (RCBD) with 3 replications, grown under normal conditions. Analysis of variance revealed that both sources of variation i.e. GCA and specific combining ability (SCA), were significant with regard to all the studied traits. Boxplot revealed that hybridization creates sufficient genetic variation for all the traits. Based on the magnitude of GCA effects, genotypes NW 5054, UP 262, and HD 2285 have been observed as significant combiners for the trait grain yield/plant. Estimates of SCA and *per se* performance, cross K 9162 × WR 544 followed by UP 262 × HD 3086, UP 262 × WR 544, HD 2285 × PBW 226, and DBW 187 × NW 5054, were the best significant specific combiners for grain yield/plant. The best heterosis crosses were UP 262 × HD 3086, K 9162 × WR 544, UP 262 × HD 2285, UP 262 × WR 544, HD 2285 × PBW 226, and DBW 187 × NW 5054, which exhibited superior heterosis over both better and mid parents for grain yield per plant. These parents and hybrids can be used further for crop improvement.

Keywords: Bread wheat, Combining ability, Diallele, Heterosis

Wheat (*Triticum aestivum* L.) is an important food crop to ensure both food and nutritional security globally. It is an allohexaploid ($2n=6x=42$) crop that belongs to the Poaceae family. The global wheat production stands at approximately 785 million metric tonnes, covering 15 million hectares (USDA 2022). India produced an average yield of 35.87 q/ha and cultivated 31.86 million hectares during 2023–24 (MOAF&W 2023). The primary causes of the yield plateau in crop production are changes in climatic patterns and limited area under cultivation (Aziz and Masmoudi 2024). Scientific and technological interventions in crop production for the development of high-yielding varieties (HYV) necessitate the doubling of crop production to meet the needs of the ever-growing population (Campbell *et al.* 2023). Identifying genotypes with superior genetic traits is essential for developing HYV that can meet increasing global

demand (Kalhoro *et al.* 2015). Heterosis or hybrid vigour, can significantly boost wheat yield by 3.5–15%, playing a vital role in increasing the overall production (Gowda *et al.* 2012, Longin 2016). Investigating heterotic patterns is critical for optimizing hybrid combinations in breeding programmes, though challenges in hybrid seed production limit practical application. Recent studies indicate that wheat hybrids developed using specific combining abilities have shown yield increments between 10–20% compared to superior pure lines, underscoring hybridization's potential for wheat production. Combining ability can be estimated using mating designs such as diallel, triallel, quadriallel, line × tester, etc. In the current investigation, for the estimation of genetic components, general combining ability (GCA), specific combining ability (SCA), and their effects, half diallel mating design has been used, preferably over other mating designs, as it evaluates all parental cross combinations with higher accuracy and reliability (Hayman 1954 and Griffing 1956). Effective breeding strategies rely on understanding the genetic mechanisms driving crop yield traits and their overall contribution to yield potential (Salem *et al.* 2020). GCA and SCA are indispensable tools for

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understanding gene action related to agronomic traits (Rojas and Sprague 1952). GCA provides insights into genotypic performance across multiple cross combinations, informing phenotypic selection (Longin *et al.* 2013). SCA indicates variations in specific crosses that occur owing to epistasis and dominance variance, is used for discovering suitable hybrid combinations for heterosis breeding (Kumari *et al.* 2015). The current study investigated the results of heterosis, GCA, and SCA in 45 F₁s cross combinations of 10 wheat varieties. The goal was to identify superior genotypes with strong GCA and crosses with the potential to significantly enhance wheat yields in the future.

MATERIALS AND METHODS

The present study was carried out during winter (*rabi*) season 2020–21 and 2021–22 at Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh. The experiment was conducted with 10 parents to produce 45 hybrids using half diallel mating design, while evaluation of the same 10 parents and 45 hybrids using randomized complete block design (RCBD) with 3 replications, grown under normal conditions by maintaining row-to-row distances of 20 cm.

Materials: Ten genotypes that were diverse in traits like disease resistance, yield potential, abiotic stress tolerance and quality parameters (Gupta *et al.* 2018) were selected as parents for hybridization (Table 1).

Observations: Plot-based observations included days to maturity (DTM) and days to 50% flowering (DTF), recorded from days to sowing. Data pertaining to plant height (PH), effective tillers/plant (TPP), spike length (SL), numbers of spikelet/spike (SPS), grains/spike (GPS), 1000-grain weight (TW), biological yield (BY), grain yield/plant (GY), and harvest index (HI) were collected from each of the three replications from five randomly selected plants.

Biometrical analysis: The combining ability estimations have been determined using Griffing's (1956) Method 2 Model I. The per cent increase and decrease of hybrids to mid parents and better parents were observed to estimate mid and better parent heterosis.

The magnitude of heterosis of calculated with the help of the formula given below:

$$\text{Heterobeltiosis: Heterosis over better parent (\%)} = \frac{F_1 - BP}{BP} \times 100$$

where BP, Value of the better parent.

$$\text{Relative heterosis: Heterosis over mid parent (\%)} = \frac{F_1 - MP}{MP} \times 100$$

where MP, Value of the mid parent.

The output data of boxplot analysis of 1000-seed weight and grain yield/plant (Fig. 1) was visualised using Microsoft Excel. The result of parents and cross-compatibility data for grain yield/plant has been depicted as a heatmap (Fig. 2) with the help of heatmap maker (<http://www.heatmapper.ca/pairwise/>).

RESULTS AND DISCUSSION

Analysis of variance: ANOVA results showed highly significant GCA and SCA for all traits studied. Grain yield, a complex trait, had significant GCA (2.71) and SCA (2.68) sources of variation. 1000-seed weight, a key yield-enhancing trait, GCA (10.96) and SCA (7.13) were also significant. Non-additive gene action was indicated as the observed ratio of GCA/SCA variance less than unity revealed the presence of greater SCA variance compared to GCA variance (Table 2). These findings of the current study revealed both additive and non-additive gene action, as their GCA and SCA were significant for all traits, which correlated with previous studies given by Tiwari *et al.* (2017), Abas *et al.* (2018), Nagar *et al.* (2018), Khokhar *et al.* (2019), Tayade *et al.* (2019) and Nageshwar *et al.* (2021). Similarly, the other key findings, such as the observed GCA/SCA variance ratio being less than unity for all the traits, were in accordance with the findings of Nagar *et al.* (2018), Kajla *et al.* (2022) and Abas *et al.* (2018), indicating the influence of non-additive gene action, which can be utilized in heterosis breeding.

Boxplot analysis: Boxplot analysis visualizes data variations, comparing mean, range, and median values of two parameters, i.e. 1000-seed weight and grain yield/plant (Fig. 1). The hybrids revealed a positive shift in

Table 1 Parents' details involved in crossing programmes

Parents	Pedigree	Year of notification	Salient features
PBW 343	ND/VG9144//KAL/BB/3/YACO'S'/4/VEE#5 'S'	1996	Double dwarf variety and stiff straw
DBW 187	NAC/TH.AC//3*PVN/3/ MIRLO/ BUC /4/2* PASTOR /5/K ACHU / 6/KACHU	2020	High Fe content (43.1 ppm)
RAJ 3765	HD 2402/VL639	1996	Tolerance to terminal heat
UP 262	S 308/BAJIO 66	1978	Suitable for both timely and late sown conditions
HD 2285	249/HD2150//HD 2186	1984	Tolerant to Karnal bunt, Early maturing
K 9162	K 7827/HD 2204	2001	Resistance to brown rust
NW 5054	THELIN//2*ATTILA*2/PASTOR	2014	Resistance to foliar blight and brown rust
PBW 226	C591/RN//JN/3/CHR/HD1941	1989	Tolerance to terminal heat stress
WR 544	KALYANSONA/HD 1999// HD 2204/DW 38	2005	Very early maturity and resistance to brown rust
HD 3086	DBW14/HD2733//HUW468	2014	Resistance to yellow and brown rust

Table 2 Analysis of variance of 10 parents for its combining ability estimation

SV	df	DTF	DTM	PH	TPP	SL	SPS	GPS	BY	HI	TW	GY
GCA	9	12.91**	15.99**	40.08**	1.10**	0.45**	1.36**	11.28**	25.50**	14.80**	10.96**	2.71**
SCA	45	11.28**	13.75**	13.11**	0.70**	0.41**	0.67**	9.37**	16.77**	7.18**	7.13**	2.68**
Error	108	1.65	0.68	1.89	0.05	0.05	0.19	1.78	1.23	0.82	0.76	0.12
GCA var.		0.94	1.28	3.18	0.09	0.03	0.10	0.79	2.02	1.16	0.85	0.22
SCA var.		9.63	13.08	11.22	0.65	0.36	0.48	7.58	15.54	6.36	6.37	2.57
GCA/SCA ratio		0.09	0.09	0.28	0.13	0.09	0.20	0.10	0.13	0.18	0.13	0.084

df, Degrees of freedom; DTF, Days to 50% flowering; DTM, Days to maturity; PH, Plant height; TPP, Tillers/plant; SL, Spike length; SPS, Numbers of spikelet/spike; GPS, Grains/spike; BY, Biological yield; HI, Harvest index; TW, 1000-grain weight; GY, Grain yield/plant; GCA, General combining ability; SCA, Self combining ability. ** indicates significant at 1% level of significance.

mean values of 1000-seed weight in hybrids (37.42) as compared to parents (36.52). Mean grain yield increased from 9.83 in parents to 11.55 in hybrids. The range of grain yield varied from 8.73–10.7 in parents, which increased to 9.04–14.53 in hybrids, with two positive outlier hybrids. This improvement indicates hybridization creates genetic variation, essential for crop improvement. Boxplot analysis of other traits, including 1000-seed weight and grain yield per plant, is available in Supplementary Fig. 1.

General combining ability (GCA) analysis: The GCA of a line results from additive gene action, reflecting its ability to combine genes for superior hybrids. The GCA analysis showed that, among the 10 parents, UP 262 (0.79), NW 5054 (0.42), and HD 2285 (0.28) were observed as effective combiners of grain yield/plant. For 1000-seed weight, significant parents were K9162 (1.58), DBW 187 (0.96), and NW 5054 (0.50) (Table 3).

The heatmap analysis highlights grain yield/plant of parents and its cross-compatibility for the selection of good combiners. High-yielding parents include DBW 187, RAJ 3765, NW 5054, UP 262, and PBW 343 (Fig. 2a). A correlation heatmap of the parents revealed that PBW 226 has high cross-compatibility with DBW 187, RAJ 3765, and NW 5054, whereas the parent WR 544 indicated moderate compatibility with DBW 187, RAJ 3765, and NW 5054. Therefore, the previously mentioned cross combinations can be suitable for heterosis breeding (Fig. 2b).

Specific combining ability (SCA) analysis: Combining ability is a main parameter for the selection of superior parental combinations in crop improvement for yields. High GCA of parents does not necessarily show high SCA in all cross combinations. Thus, it is important to estimate SCA, which can be defined as the cross combination of a hybrid having superior or inferior performance compared to the parents. The cross combination having high SCA confirms the presence of a non-additive type of gene action. The SCA of grain yield/plant for 45 crosses is showcased in Supplementary Table 1. The 5 best hybrids showing the highest estimates of SCA for grain yield/plant were K 9162 × WR 544 (3.82), followed by UP 262 × HD 3086 (3.68), UP 262 × WR 544 (3.01), HD2285 × PBW 226 (2.90), and DBW 187 × NW 5054 (2.76). The SCA values for all traits of 45 crosses (Supplementary Table 2).

Heterosis analysis: Heterosis is a key objective for developing high-yielding genotypes, driven by heterozygosity in specific cross combinations (Al-Mamun *et al.* 2022). The heterosis values for the dependent trait grain yield/plant across 45 hybrids showed a range from -11.63% (lowest) to 61.55% (highest) over the mid parent and -15.51% to 58.48% compared to the better parent (Supplementary Table 1). Out of the 45 hybrids, 32 observed highly significant and positive heterosis compared to the mid parent. Similarly, 24 hybrids displayed positive significant heterosis over better parent, with best crosses such as UP

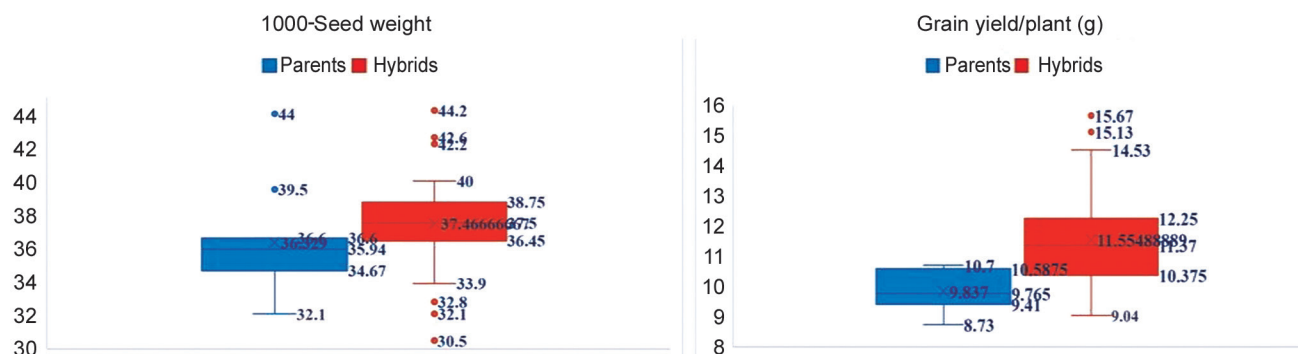


Fig. 1 Boxplot analysis of parents versus hybrids represented for two traits.

Cross “x”, Mean; Horizontal line inside the box, Median; and Whisker line, Range with outlier (blue and red dots).

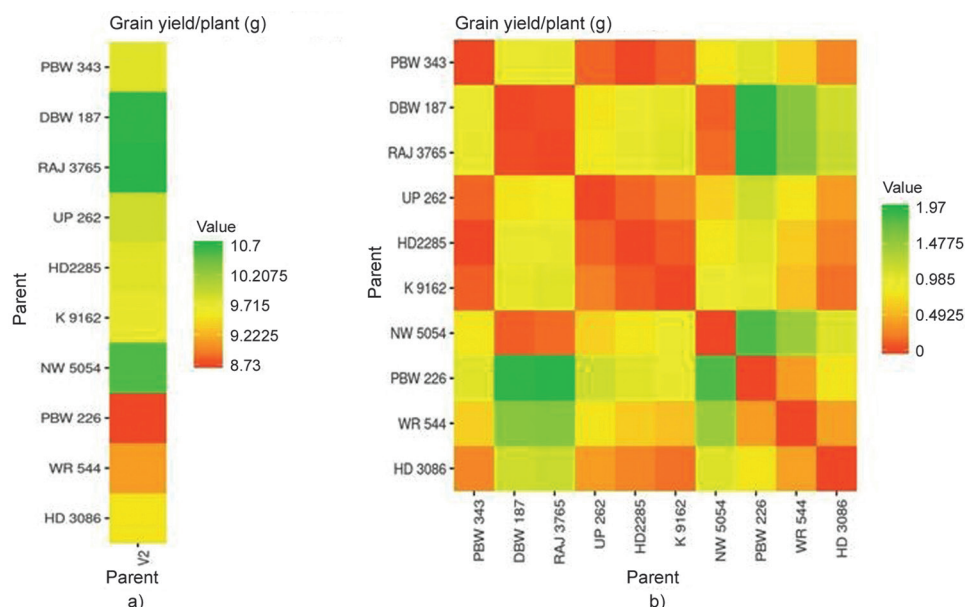


Fig. 2 Heatmap representing a) grain yield/plant and b) cross compatibility of 10 parents for grain yield/plant.

The colour gradient for lowest, moderate and highest value represented in red, yellow and by dark green colours, respectively.

Table 3 Representation of GCA effects of the ten parents involved in the hybridization.

Parents/Traits	DTF	DTM	PH	TPP	SL	SPS	GPS	BY	HI	TW	GY
P1 (PBW 343)	1.29 **	1.12 **	-3.33 **	0.1	-0.31 **	0.14	0.27	-1.21 **	0.94 **	0.49 *	-0.14
P2 (DBW 187)	-2.12 **	-2.54 **	1.14 **	0.06	0.05	0.41 **	0.97 **	1.67 **	-1.52 **	0.96 **	0.11
P3 (RAJ 3765)	0.29	-0.63 **	-0.59	0.03	0.03	-0.03	-0.43	-0.39	-2.21 **	-0.88 **	-0.76 **
P4 (UP 262)	0.96 **	1.51 **	1.43 **	0.50 **	0.44 **	0.30 *	1.33 **	2.23 **	-0.08	-0.2	0.79 **
P5 (HD 2285)	-0.71 *	-0.18	-0.84 *	-0.04	-0.08	-0.59 **	-1.56 **	-0.13	1.00 **	-0.22	0.28 **
P 6 (K 9162)	-0.12	0.15	-0.79 *	-0.44 **	0.11	-0.16	-0.38	0.08	-0.08	1.58 **	-0.02
P7 (NW 5054)	1.18 **	1.09 **	2.61 **	-0.54 **	0.03	0.50 **	1.42 **	0.55	0.76 **	0.50 *	0.42 **
P8 (PBW 226)	0.21	0.21	-1.13 **	0.03	-0.08	-0.23	-0.63	-3.01 **	1.12 **	-1.51 **	-0.73 **
P9 (WR 544)	-0.62	-0.52 *	2.30 **	0.04	-0.08	-0.12	-0.46	0.52	-0.25	0.32	0.09
P10 (HD 3086)	-0.37	-0.21	-0.79 *	0.27 **	-0.11	-0.23	-0.53	-0.33	0.32	-1.03 **	-0.03

*, ** indicates significant at 5% and 1% level of significance, respectively.

DTF, Days to 50% flowering; DTM, Days to maturity; PH, Plant height; TPP, Tillers/plant; SL, Spike length; SPS, Numbers of spikelet/spike; GPS, Grains/spike; BY, Biological yield; HI, Harvest index; TW, 1000-grain weight; GY, Grain yield/plant.

262 × HD 3086 (58.48%), K 9162 × WR 544 (56.55%), and UP 262 × WR 544 (52.95%). The findings of Gul *et al.* (2015), Mahpara *et al.* (2015), Ahmad *et al.* (2016), Kumar (2017), Motawea (2017), Shah *et al.* (2018), Sharma *et al.* (2018), Shrief *et al.* (2019), Kumar *et al.* (2019) and (2020) align with our current investigation as they have reported significant heterosis over mid and better parent in different hybrids for the grain yield trait. Data pertaining to heterosis and the best heterotic hybrids for all traits are presented (Supplementary Table 3 and Supplementary Table 4). These combinations require special attention for future breeding programmes to harness heterosis effectively.

The present investigation evaluated heterotic effects and combining ability among 10 parental genotypes using

a half-diallel mating design. Box plot analysis indicated that hybridization produced diverse genotypes across all traits studied while comparing hybrids to their parents. GCA analysis highlighted UP 262, NW 5054, and HD 2285 as excellent combiners for grain yield. For exploiting heterosis, SCA effects identified significant crosses such as K 9162 × WR 544, UP 262 × HD 3086, UP 262 × WR 544, HD 2285 × PBW 226, and DBW 187 × NW 5054 as promising for grain yield. Notably, UP 262 × HD 3086, K 9162 × WR 544, UP 262 × HD 2285, UP 262 × WR 544, and HD 2285 × PBW 226 showed highly

significant heterosis over both better and mid parents, indicating their potential for future breeding programmes.

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