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IDENTIFICATION OF HETEROTIC CROSS COMBINATIONS FOR GRAIN YIELD AND ASSOCIATED TRAITS IN BREAD WHEAT (*Triticum aestivum* L.)

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Wheat (*Triticum aestivum* L.), a critical staple for over 30% of the global population, is cultivated annually across approximately 240 million hectares. With global production surpassing 781 million metric tons, India plays a significant role in wheat cultivation (FAO, 2023). Addressing the escalating food demands driven by a growing population necessitates continual advancements in wheat production. Hybrid breeding, which exploits heterosis—the superior performance of offspring from genetically diverse parents—presents a promising strategy. To optimize heterosis, it is essential to categorize lines into distinct genetic pools. Empirical evidence indicates a positive correlation between parental genetic diversity and the level of heterosis in their hybrid progeny (Melchinger, 1999; Reif *et al*., 2005). A comprehensive understanding of the underlying genetic mechanisms is critical for developing complementary lines that exhibit high heterotic potential (Whitford *et al*., 2013).

The phenomenon of heterosis was initially described by Koelreuter, who associated it with outcrossing. Hybrids resulting from the combination of genetic material from separate parental sources frequently display enhanced traits relative to their inbred progenitors. Typically, F_1 hybrids exhibit improved stress tolerance, increased yields, and accelerated growth rates (Sang-Ho Chu *et al*., 2012). However, exploiting heterosis in wheat presents unique challenges due to its naturally inbreeding characteristics and historical limitations in effective fertility control methods. Although hybrid breeding has achieved significant success in crops such as maize and vegetables, its application in self-pollinated crops like wheat has been less effective, with notable exceptions such as hybrid rice in China. This reduced effectiveness is largely attributed to the lower levels of heterosis observed in these crops (Gupta *et al*., 2019).

The benefits of F_1 hybrid vigour are well-documented across various crops. In wheat, the first reports of heterosis were made by Freeman (1919), who highlighted the advantages of F_1 hybrids. The discovery of cytoplasmic male sterility and restoration in *Triticum timopheevi* in 1962 offered a potential framework for developing wheat hybrids using a three-line system (male sterile line, maintainer line, and restorer line) (Yingbin Nie *et al*., 2019). Nevertheless, the magnitude and direction of heterosis in self-pollinated crops like wheat significantly influence the practicality of harnessing this phenomenon (Singh *et al*., 2004). This study aims to assess the degree of heterosis in bread wheat to identify promising crosses for future breeding programs.

The experiment was conducted at the crop research farm of Janta Vedic College, Baraut (Baghpat), Uttar Pradesh (UP). During the *rabi* season of 2017-18, ten parental wheat lines—HD2967, WH-1105, UP 2748, UP 2565, UP 2572, UP 2628, UP 2526, HD 3059, HD 3086, and WH 1021—were crossed in a half-diallel fashion, resulting in 45 F_1 hybrids (excluding reciprocals). The F_1 progeny and parental lines were evaluated in the subsequent *rabi* season of 2018-19 using a randomized block design with three replications. Each plot consisted of two 1-meter rows per entry in each replication.

Observations were recorded for days to heading and days to maturity across the entire plot. For additional traits, data were collected from five randomly selected plants per entry. The specific traits measured were: Flag Leaf Area (cm²): Five flag leaves were collected from randomly selected plants during the morning, ensuring they were fully turgid. The leaf area was calculated using the method described by Muller (1991). Plant Height at Maturity (cm): The height of the plant, excluding awns, was measured from the soil surface to the tip of the spike at approximately half grain filling. Number of Productive Tillers per Plant: Recorded prior to harvest, the number of productive tillers per plant was counted for each selected plant within a replicate. Spike Length (cm): The length of the main tiller's spike, excluding awns, was measured from the base of the spike to the tip of the uppermost spikelets. Number of Grains per Spike: The average number of grains per spike was determined by counting the seeds from randomly selected spikes. 1000-Grain Weight (g): A sample of one thousand grains from each selection was weighed using an electronic balance. Biological Yield per Plant (g): The total dry weight of five plants, excluding roots, was measured to estimate their biological yield. The average dry weight was used for subsequent statistical analysis.

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Grain Yield per Plant: The average grain yield per plant (in grams) was determined by harvesting, threshing, cleaning, and weighing all ear heads from five randomly selected plants together. The total weight of the grains was then divided by the number of plants to compute the average grain yield per plant.

Harvest Index: The harvest index represents the ratio of seed yield per plant to biological yield per plant, expressed as a percentage. It is calculated using the following formula:

$$\text{Harvest Index (\%)} = \frac{\text{Grain yield per plant}}{\text{Biological yield per plant}} \times 100$$

The mean values for each characteristic were used for subsequent analysis. To statistically evaluate differences between parental lines and their F_1 hybrids, an analysis of variance (ANOVA) was performed following the methods described by Gomez and Gomez (1984). Additionally, a diallel analysis using Griffing's Method-2 Model-1 (Griffing, 1956) was employed to assess genetic variation among the parents and their F_1 hybrids, comprising n(n-1)/2 crosses. This approach, as outlined by Singh and Chaudhary (1979), facilitated the estimation of both additive and non-additive genetic variances and their impacts on the various traits.

Estimation of heterosis: The percent increase (+) or decrease (−) of each F_1 hybrid relative to both the mid-parent and the better parent was calculated to assess heterotic effects for all parameters. Heterosis, defined as the superior performance of hybrids compared to their parents, was estimated over the mid-parent and better parent (heterobeltiosis) using the methodologies outlined by Matzinger *et al.* (1962) and Fonseca and Patterson (1968).

$$\text{Heterosis over the Mid parent (MP\%)} = \frac{(F_1 - MP)}{MP} \times 100$$

$$S.E\ (F_1 - MP) = \frac{\sqrt{3Me}}{2r}$$

$$\text{MP = mid parent value of the particular } F_1 \text{ cross} = \frac{(P_1 - P_2)}{2}$$

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

$$SE\ (F_1 - BP) = \frac{\sqrt{2Me}}{r}$$

where,

Me =error mean squares for parents and F_1s

$$\text{MP = mean parent value} = \frac{(P1 - P2)}{2}$$

P_1= mean performance of parent one

P_2 = mean performance of parent two;

BP = mean better-parent value;

r = number of replications;

Significance of MP and HB were tested by't' test using SE values in all the traits.

This study assessed heterosis to identify promising cross combinations for future hybrid wheat breeding programs. All eleven traits analyzed demonstrated significant levels of desirable heterosis, surpassing both mid-parent and better-parent values, as detailed in Table 1. Of the 45 F_1 hybrids evaluated, 17 exhibited significant positive heterosis (H), while 12 showed significant positive heterobeltiosis (HB), as presented in Table 2.

Days to heading: The mid-parent heterosis for the traits analyzed ranged from -0.19% for the cross UP 2572 × HD 3059 to 4.90% for the cross WH 1105 × HD 3086. Correspondingly, the better-parent heterosis (heterobeltiosis) ranged from -0.38% for UP 2572 × UP 2526 to 2.70% for HD 2967 × UP 2628. Negative heterosis for days to heading was observed in only four crosses relative to the mid-parent, while fourteen crosses displayed significant negative heterobeltiosis values. Notably, the cross UP 2565 × HD 3059 exhibited the highest positive mid-parent heterosis, whereas UP 2748 × WH 1021 demonstrated the most significant negative heterobeltiosis. These crosses with negative heterotic values for days to heading may be particularly valuable for developing wheat cultivars with shorter maturation periods. These observations align with similar findings reported in the literature by Jaiswal *et al.* (2010), Kumar *et al.* (2015), Garg *et al.* (2015), Desale and Mehta (2015), and Kumar *et al.* (2017).

Days to maturity: Mid-parent heterosis for days to maturity ranged from -0.00% for the cross WH 1105 × UP 2628 to 2.45% for HD 2967 × UP 2526. Similarly, better-parent heterosis varied from -0.54% for UP 2748 × UP 2565 to 2.39% for HD 2967 × HD 3059. Significant negative heterosis values for days to maturity were observed in twenty-two crosses for mid-parent heterosis and thirty-three crosses for better-parent heterosis. The cross UP 2748 × WH 1021 exhibited the most negative heterosis for both mid-parent and better-parent values, suggesting a dominance for earlier maturity. These findings regarding negative heterosis for days to maturity are consistent with previous studies by Devi *et al.* (2013), Lal *et al.* (2013), and Ahmad *et al.* (2016). In wheat breeding, early heading and maturity are considered beneficial traits for enhancing crop performance.

Plant height (cm)

During the Green Revolution, plant breeders

Table 1. Heterosis range, heterosis mean, number of desirable hybrids, and best hybrids and mid parent for yield and yield contributing traits in wheat

Traits	Heterosis hybrid	Heterosis range (%)	No. of desired hybrids	Best hybrids
Days to heading	Mid Parent (MP)	-0.19 to 4.90	4	UP2565 XHD3059 (-2.14)
	Better Parent (BP)	-0.38 to 2.70	14	UP2748 X WH1021 (-2.33)
Days to maturity	Mid Parent (MP)	-0.00 to 2.45	22	UP 2748 x WH 1021 (-4.01)
	Better Parent (BP)	-0.54 to 2.39	33	UP 2748 x WH 1021 (-4.27)
Plant height (cm)	Mid Parent (MP)	-0.33 to 5.44	33	HD 2967 x UP 2628 (-14.11)
	Better Parent (BP)	-0.91to 2.37	40	HD 2967 X UP 2572 (-14.79)
Productive tillers/ plants	Mid Parent (MP)	-1.97 to 35.57	13	UP 2748 x HD 3059 (35.57)
	Better Parent (BP)	-2.14 to 25.82	6	UP 2748 x HD 3059 (25.82)
Flag leaf area (cm)	Mid Parent (MP)	-1.32 to 36.27	23	UP 2565 x UP 2526 (36.27)
	Better Parent (BP)	-0.57 to 28.18	17	UP 2565 x UP 2526 (28.18)
Spike length (cm)	Mid Parent (MP)	-0.03 to 15.32	19	UP 2748 x UP 2572 (15.32)
	Better Parent (BP)	-0.59 to 12.31	10	UP 2748 x UP 2572 (12.31)
Grains per spike	Mid Parent (MP)	-0.17 to 22.71	21	UP 2748 XUP 2565 (22.71)
	Better Parent (BP)	-1.55 to 20.11	11	UP 2748 x WH 1021 (20.11)
1000-grain weight	Mid Parent (MP)	-2.09 to 13.10	20	UP 2748 x HD 3059 (13.10)
	Better Parent (BP)	-0.01 to 13.05	12	UP 2748 x HD 3059 (13.05)
Biological yield/ Plant	Mid Parent (MP)	-1.16 to 53.38	13	UP 2748 x HD 3059 (53.38)
	Better Parent (BP)	-1.53 to 45.47	10	UP 2748 x HD 3059 (45.47)
Harvest Index	Mid Parent (MP)	-0.10 to 20.72	17	UP 2572 x WH 1021 (20.72)
	Better Parent (BP)	-0.64 to 19.30	10	UP 2748 x HD 3059 (19.30)
Grain yield/ Plants	Mid Parent (MP)	-0.24 to 58.89	17	UP 2748 x HD 3059 (58.89)
	Better Parent (BP)	-0.15 to 56.64	12	UP 2748 x HD 3059 (56.64)

focused on modifying wheat genetics to reduce plant height, leading to the widespread adoption of short-statured cultivars. In wheat breeding, negative estimates of heterosis and heterobeltiosis for plant height are considered favourable, as they indicate dwarfism—a desirable trait for enhancing stability and yield. Mid-parent heterosis for plant height ranged from -0.33% for the cross UP 2572 × WH 1021 to 5.44% for UP 2572 × UP 2526. Similarly, better-parent heterosis ranged from -0.91% for UP 2572 × WH 1021 to 2.37% for UP 2748 × HD 3086.

Thirty-three crosses exhibited significant negative values for both mid-parent and better-parent heterosis for plant height. Among these, the cross HD 2967 × UP 2628 displayed the most negative mid-parent heterosis, while the cross HD 2967 × UP 2572 showed the most significant negative heterobeltiosis (better-parent heterosis). These observations align with findings from previous studies by Beche *et al.* (2013), Singh *et al.* (2013), and Kumar *et al.* (2021).

Productive tillers per plant: Mid-parent heterosis for productive tillers per plant ranged from -1.97% for HD 2967 × UP 2572 to 35.57% for UP 2748 × HD 3059, while better-parent heterosis ranged from -2.14% for HD 2967 × UP 2572 to 25.82% for UP 2748 × HD 3059. Thirteen crosses exhibited significant positive heterosis over the mid-parent, and six crosses showed significant positive heterosis over the better-parent. Notably, the cross UP 2748 × HD 3059 stood out as the superior combination, demonstrating significant positive heterotic values for both mid-parent and better-parent heterosis. This cross also exhibited the highest positive heterobeltiosis, indicating its exceptional performance for productive tillers per plant. Identifying similar hybrids with these advantageous traits through single-plant selection is promising. These findings are consistent with previous research by Garg *et al.* (2015), Singh *et al.* (2016), and Kumar *et al.* (2016).

Spike length (cm): Mid-parent heterosis for spike length ranged from -0.03% for UP 2572 × WH 1021

Table 2. **Cross combinations exhibiting significant desirable mid and better parents' heterosis**

S.N	Crosses	Mid parent value	Better parent value	Traits	Combiner	Mode of gene action
1	UP 2748 x HD 3059	58.879	56.64	GPP, BYPP, TGW, GPS, SL, PTPP, FLA DM and PH.	Good x Poor	Non fixable
2	UP 2748 x UP 2565	52.24	47.53	GPP, GPS, SL, BYPP, HI, PTPP, FLA, and PH	Good x Good	Fixable type
3	UP 2748 x UP 2628	41.95	34.3	GPP, SL, GPS, TGW,BYPP, HI, FLA, DH, DM and PH	Good x Poor	Non fixable
4	UP 2565 x WH 1021	26.65	26.63	GPP, GPS, TGWBYPP, HI, DM PH and PTPP	Good x Poor	Non fixable
5	WH 1105 x UP 2526	31.21	20.52	GPP, SL, GPS, TGWBYPP, HI, DM PH, PTPP, and FLA	Good x Poor	Non fixable
6	UP 2748 x UP 2572	30.29	20.13	GPP, SL, GPS, HI, PH	Good x Poor	Non fixable
7	WH 1105 x WH 1021	32.23	18.73	GPP,, SL, TGWBYPP, HI, PH, PTPP and FLA	Good x Poor	Non fixable
8	UP 2565 x HD 3086	33.58	11.46	GPP, GPS, TGW, BYPP and Harvest Index.	Good x Good	Fixable type
9	HD 3059 x WH 1021	17.07	15.07	GPP, HI, and PH	Poor x Poor	Non fixable
10	UP 2565 x UP 2526	12.64	9.88	GPP, HI, PH and FLA	Good x Poor	Non fixable
11	UP 2565 x UP 2572	15.3	9.52	GPP, SL, PH and FLA	Good x Poor	Non fixable
12	UP 2565 x HD 3059	7.54	5.68	GPP, GPS, TGW,BYPP, DM PH, PTPP and FLA	Good x Poor	Non fixable

where, DH= Days to heading, DM = Day to maturity, PH = Plant height, FLA = Flag leaf area, PT = Productive tillers per plant, SL = Spike length, GS= Grains per spike, TGW = Thousand grain weight, BY = Biological yield per plant, HI = harvest index

to 15.32% for UP 2748 × UP 2572. Similarly, better-parent heterosis varied from -0.59% for HD 2967 × UP 2748 to 12.31% for UP 2748 × UP 2572. Spike length is a crucial yield component that positively influences productivity, making significant heterosis for this trait particularly desirable. In this study, nineteen crosses exhibited positive and significant mid-parent heterosis values, while ten crosses showed significant positive heterosis over the better parent. Notably, the cross UP 2748 × UP 2572 demonstrated the highest level of heterosis, exceeding both mid-parent and better-parent values, and emerged as the most promising candidate due to its significant heterotic response. These findings align with previous studies by Ahmad *et al.* (2016), Ahmad and Jaiswal (2016), and Kumar *et al.* (2017).

Flag leaf area, (cm): Mid-parent heterosis for flag leaf area ranged from -1.32% for HD 2967 × UP 2572 to 36.27% for UP 2565 × UP 2526. Similarly, better-parent heterosis varied from -0.57% for UP 2572 × WH 1021 to 28.18% for UP 2565 × UP 2526. Among the crosses evaluated, twenty-three exhibited positive and significant heterosis exceeding both mid-parent and better-parent values, while seventeen crosses showed significant positive heterosis surpassing only mid-parent values. Flag leaf area is a critical trait for yield enhancement, as it contributes significantly to photosynthesis. The cross UP 2565 × UP 2526 emerged as the most promising combination, demonstrating the highest positive and significant heterotic values for both mid-parent and better-parent comparisons. This finding is consistent with previous studies by Jaiswal *et al.* (2018), Ibrahim *et al.* (2020), and Singh *et al.* (2020). The superior performance of this cross highlights its potential to enhance grain quality and increase photosynthetic efficiency, as the flag leaf is responsible for over 70% of a plant's photosynthesis during the grain-filling period.

Grains per spike: Mid-parent heterosis for the number of grains per spike ranged from -0.17% for HD 2967 × UP 2565 to 22.71% for UP 2748 × UP 2565. Similarly, better-parent heterosis varied from -1.55% for UP 2565 × UP 2628 to 20.11% for UP 2748 × WH 1021. Among the evaluated crosses, twenty-one exhibited positive and highly significant heterosis exceeding both mid-parent and better-parent values, while eleven crosses showed positive and highly significant heterosis exceeding only mid-parent values. Notably, the crosses UP 2748 × UP 2565 and UP 2748 × WH 1021 demonstrated significant positive heterosis for the number of grains per spike relative to both mid-parent and better-parent values. These findings are in agreement with previous studies by Kalhoro *et al.* (2015), Nawara *et al.* (2016), Yadav *et al.* (2017), and Kumar *et al.* (2018).

1000-grain weight: Mid-parent heterosis for 1000-grain weight ranged from -2.09% for HD 2967 × UP 2748 to 13.10% for UP 2748 × HD 3059, while better-parent heterosis ranged from -0.01% for UP 2526 × HD 3059 to 13.05% for UP 2748 × HD 3059. Twenty crosses exhibited positive and significant heterosis exceeding both mid-parent and better-parent values, while twelve crosses showed positive and significant heterosis surpassing only the mid-parent values. Notably, the cross UP 2748 × HD 3059 demonstrated a substantial positive heterotic value for 1000-grain weight, exceeding both mid-parent and better-parent values. This cross also recorded the highest positive and significant heterosis among all the crosses studied, highlighting the importance of 1000-grain weight as a critical yield component. These findings are consistent with previous studies by Kumar *et al.* (2017) and Nagar *et al.* (2019).

Biological yield per plant: Mid-parent heterosis for biological yield per plant (BYPP) ranged from -1.16% for HD 2967 × UP 2565 to 53.38% for UP 2748 × HD 3059, while better-parent heterosis varied from -1.53% for UP 2572 × UP 2628 to 45.47% for UP 2748 × HD 3059. Thirteen crosses exhibited significant positive heterosis exceeding both mid-parent and better-parent values, and twelve crosses showed significant positive heterosis exceeding only the mid-parent values. Notably, the cross UP 2748 × HD 3059 demonstrated a substantial heterotic effect for BYPP, surpassing both mid-parent and better-parent values. This cross emerged as the most promising due to its highest positive and significant heterotic value. These results are consistent with findings reported by Kant *et al.* (2011), Kumar *et al.* (2016), and Upadhyay *et al.* (2020).

Harvest index: Mid-parent heterosis for harvest index (HI) ranged from -0.10% for HD 2967 × WH 1021 to 20.72% for UP 2572 × WH 1021, while better-parent heterosis ranged from -0.64% for UP 2748 × WH 1021 to 19.30% for UP 2572 × WH 1021. Seventeen crosses exhibited significant positive heterosis exceeding both mid-parent and better-parent values, while ten crosses showed significant positive heterosis surpassing only mid-parent values. Notably, the crosses UP 2572 × WH 1021 and UP 2748 × HD 3059 demonstrated the highest magnitudes for harvest index, exceeding both mid-parent and better-parent values. Harvest index is a critical trait contributing to yield. These findings are consistent with previous reports by Rajend Kandalkar (2013a), Kumar *et al.* (2016), and Rajput and Kandalkar (2018b).

Grain yield per plant

Grain yield is the most crucial trait in wheat and is complex to manipulate genetically due to its control by quantitative gene action. Mid-parent heterosis for grain yield per plant ranged from -0.24% for UP 2748 × WH 1021 to 58.89% for UP 2748 × HD 3059, while better-parent heterosis ranged from -0.15% for UP 2526 × HD 3059 to 56.64% for UP 2748 × HD 3059. Seventeen crosses exhibited significant positive heterosis over the mid-parent values, and twelve crosses showed significant positive heterosis exceeding better-parent values. Notably, the crosses UP 2748 × HD 3059 and UP 2748 × UP 2565 demonstrated substantial heterosis, attributable to their superior performance in biological yield per plant, 1000-grain weight, grains per spike, spike length, productive tillers per plant, and flag leaf area.

The findings of this study highlight the potential for identifying transgressive segregants among these crosses, which could lead to the development of high-yielding wheat cultivars with desirable quality attributes. Similar results have been reported by Desale and Mehta (2013), Kalhoro *et al.* (2015), Singh *et al.* (2018), Sharma *et al.* (2018), Kumar *et al.* (2019), Nagar *et al.* (2019), Kajla *et al.* (2020), and Choudhary *et al.* (2022). Evaluating hybrids in relation to their parents (heterobeltiosis) is essential for determining the extent of commercially exploitable heterosis and assessing whether specific parental combinations yield the most promising transgressive segregants.

CONCLUSION

This study effectively identified promising heterotic crosses among 45 F_1 hybrids derived from 10 parental lines, demonstrating significant heterosis for all examined traits. The cross UP 2748 × HD 3059 stood out with the highest heterobeltiosis of 58.89% for grain yield per plant, marking it as a particularly valuable candidate for future breeding programs. This research opens several promising avenues for further exploration:

- **Development of High-Yielding Varieties:** The identified heterotic crosses, such as UP 2748 × HD 3059, UP 2565 × HD 3059, and UP 2748 × WH 1021, can be leveraged in breeding programs to create new, high-yielding wheat varieties. These crosses offer a strong foundation for enhancing yield potential in future cultivars.
- **Exploitation of Heterosis for Additional Traits:** The study also uncovered significant heterosis for various yield-contributing components, including days to heading, plant height, and number of grains per spike. This suggests opportunities to breed

varieties that not only achieve high yields but also exhibit desirable agronomic traits, improving overall crop performance.

- **Identification of Transgressive Segregants:** The significant heterosis observed indicates the potential for transgressive segregants within the F_2 population derived from the identified crosses. These transgressive segregants, which display superior traits beyond those of their parent lines, can be further selected and developed into homozygous lines, potentially leading to new varieties with exceptional characteristics.
- **Marker-Assisted Breeding:** Future research could incorporate molecular markers associated with heterotic loci into breeding programs. By using marker-assisted selection, breeders can more efficiently identify and develop superior lines with desirable traits at the seedling stage, accelerating the breeding process and enhancing the precision of trait selection.

Overall, this study provides a solid foundation for advancing wheat breeding efforts, highlighting several strategic directions for improving yield and agronomic quality in wheat cultivars.

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