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ESTIMATION OF HETEROSIS FOR YIELD, YIELD-ATTRIBUTING TRAITS AND STARCH CONTENT IN DOUBLED HAPLOID DERIVED CROSSES OF MAIZE (*Zea mays* L.)

N. Lavanya[1], G. Usharani[2*], D. Sravani [3] and K. Lakshmi Prasanna[4]
[1]Department of Genetics and Plant Breeding, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India.
[2]Plant Breeding, Agricultural Research Station, Karimnagar, PJTAU, Telangana, India.
[3]Plant Breeding, Maize Research Unit, Agricultural Research Institute, Rajendranagar, PJTAU, Hyderabad, Telangana, India.
[4]Seed Science and Technology, Seed Research and Technology Centre, Rajendranagar, PJTAU, Hyderabad, Telangana, India.
*Corresponding author E-mail: ushagenetics@gmail.com
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ABSTRACT

Heterosis breeding remains a proven route to improving grain yield in maize, and doubled haploid (DH) technology, which fixes fully homozygous lines in a single generation, sharpens heterosis estimates by removing residual heterozygosity from parental material. This study estimated mid-parent heterosis, heterobeltiosis and standard heterosis for grain yield, its component traits and starch content in 40 DH-derived single-cross hybrids from 20 DH lines and 2 testers via a Line × Tester design. The hybrids, with five commercial checks DHM-206, DHM-182, NK6802, DHM-117 and Karimnagar Makka-1 were evaluated in a randomized block design with two replications at Agricultural Research Station, Karimnagar, PJTAU, during kharif 2025. Standard heterosis was estimated independently against each of the five commercial checks rather than using a single reference hybrid. Mid-parent heterosis and heterobeltiosis were significant and positive for grain yield in all 40 crosses (80.67–318.05% and 69.13–308.10%, respectively), DH23 × KML-137 highest on both bases. Standard heterosis for grain yield depended strongly on the check used: none of the crosses exceeded the two highest-yielding checks, DHM-206 and DHM-182, but five crosses DH11 × KML-87, DH17 × KML-87, DH22 × KML-137, DH23 × KML-87 and DH23 × KML-137 significantly exceeded the three lower-yielding checks, NK6802, DHM117 and Karimnagar Makka-1, up to 26.78% (DH22 × KML-137 over NK6802). Standard heterosis for starch content remained non-significant against all five checks, with the highest value of 1.46% recorded in DH8 × KML-137 over Karimnagar Makka-1. The crosses DH22 × KML-137, DH23 × KML-137, DH11 × KML-87, DH17 × KML-87, DH23 × KML-87 (grain yield), DH13 × KML-137 (earliness) and DH8 × KML-137 (starch) were the most promising, meriting further evaluation.

***Keywords* :** Maize; *Zea mays* L.; doubled haploid; heterosis; heterobeltiosis; standard heterosis; multiple checks; line × tester; starch content; grain yield.

Introduction

Maize (*Zea mays* L.) is a cross-pollinated cereal whose commercial cultivation rests almost entirely on the exploitation of hybrid vigour, or heterosis, first described by Shull (1908) and attributed by Jones (1918) to the combined action of dominant genes contributed by both parents. India produced 422.81 lakh tonnes of maize from 107.28 lakh hectares in 2024–25 (PIB, 2025), with Telangana alone contributing over 30 lakh tonnes at a productivity well above the national average (PJTAU, 2025), reflecting the crop's continuing importance to the state's agrarian economy.

The precision of heterosis estimates depends on the genetic purity of the parents used to generate hybrids. Doubled haploid (DH) technology fixes completely homozygous lines in a single generation, unlike the residual heterozygosity that persists even after several generations of conventional selfing, and this has been shown to sharpen combining ability and heterosis estimates in maize (Longin *et al*., 2007; Geiger *et al*., 2013). DH-derived hybrids have recorded standard heterosis of 30–49% over commercial checks in several tropical breeding programmes (Beyene *et al*., 2011; Sserumaga *et al*., 2016), and heterosis breeding has also been used to improve starch content, which forms about 70% of the kernel's dry weight and is central to the crop's food, feed and industrial value (Bisen *et al*., 2017; Khan *et al*., 2018).

Standard heterosis is conventionally expressed over a single 'best' commercial check, yet the magnitude, and sometimes even the sign, of standard heterosis for a given cross can change considerably depending on which commercial hybrid is chosen as that standard, particularly when several checks of differing yield potential are available. Using only one check to evaluate new hybrids may not accurately reflect their true commercial performance, as it can either overestimate or underestimate their competitiveness. Despite this, DH-derived maize crosses have rarely been evaluated for heterosis in grain yield, its component traits and starch content together under Telangana conditions, and more rarely still against more than one standard check simultaneously. The present study was therefore undertaken to estimate mid-parent heterosis, heterobeltiosis and standard heterosis the last estimated independently against each of five commercial checks for these traits in a set of doubled haploid derived single-cross hybrids of maize.

Materials and Methods

The study was conducted during kharif 2025 at the Agricultural Research Station, Karimnagar, Professor Jayashankar Telangana State Agricultural University (PJTAU). Forty single-cross hybrids, generated by crossing 20 doubled haploid lines with 2 testers in a Line × Tester mating design, were evaluated along with 5 commercial hybrids DHM-206, DHM-182, NK6802, DHM-117 and Karimnagar Makka-1 as standard checks, giving 45 entries in total. Standard heterosis was computed separately against each of the five checks for every character, so that each of the 40 crosses carries five standard-heterosis estimates per character instead of one. Rather than comparing DH-derived hybrids with only one standard genotype, the check-wise approach evaluates them against a representative set of commercial hybrids, providing insight into the effect of check selection on standard heterosis.

The 45 entries were evaluated in a randomized block design with two replications. Each entry was grown in a two-row plot of 4 m length (60 × 20 cm spacing), giving a net plot size of 4.8 m², with the recommended package of practices followed uniformly across all plots. Data were recorded on five randomly tagged, competitive plants per plot for days to 50% anthesis and silking, days to maturity, plant height (cm), ear height (cm), ear length (cm), ear girth (cm), kernel rows per ear, kernels per row, test weight (g), shelling percentage, grain yield (kg ha^{-1}) and starch content (%).

Starch content was estimated by the Anthrone method (Sadasivam and Manickam, 1992). Free sugars were removed from ground kernel samples using hot 80% ethanol, the residual starch was hydrolysed with 52% perchloric acid, and the glucose released was estimated colorimetrically at 620 nm using the anthrone reagent, with the glucose value converted to a starch equivalent using a factor of 0.9.

Data were subjected to analysis of variance appropriate to a randomized block design (Panse and Sukhatme, 1985). Heterosis was estimated following Kempthorne (1957) as:

$$\text{Mid-parent heterosis}(\%) = \frac{\overline{F}_1 - \overline{MP}}{\overline{MP}} \times 100$$

(Briggle, 1963)

$$\text{Heterobeltiosis}(\%) = \frac{\overline{F}_1 - BP}{BP} \times 100$$

(Fonseca and Patterson, 1968)

$$\text{Standard heterosis}(\%) = \frac{\overline{F}_1 - SC}{SC} \times 100$$

(Meredith and Bridge, 1972)

where $\overline{MP}$ and BP are the mid-parent and better-parent means, respectively, and SC is the mean of a standard check. Because five commercial checks were used, standard heterosis was calculated five times for every cross and character once with SC taken in turn as the mean of DHM-206, DHM-182, NK6802, DHM117 and Karimnagar Makka-1 rather than once against a single designated check. The significance of each heterosis estimate was tested using the standard error of the difference between two means and the t-test at the error degrees of freedom, at the 5% and 1% levels of probability (Panse and Sukhatme, 1985).

Results and Discussion

Analysis of the forty single-cross hybrids revealed considerable heterosis for most of the fourteen characters studied, although its magnitude and direction differed markedly between the mid parent, the better parent and the standard checks, a pattern also documented by Sharma *et al*. (2017) in a comparably designed line × tester study in maize. Mid-parent heterosis and heterobeltiosis are summarised in Table 1; standard heterosis, computed independently against each of the five commercial checks, is summarised check-by-check in Table 2.

For the phenological traits, DH13 × KML-137 was consistently the earliest of the forty hybrids on every basis of comparison, recording a mid-parent heterosis of −7.26% for days to 50% anthesis, the most negative of any cross, and heterobeltiosis of −9.45%. Standard heterosis for this trait depended on which check was used: DH13 × KML-137 was significant against three of the five checks DHM-206, DHM-182 and DHM-117 (−4.96%, P≤0.05 in each case) and highly significant against NK6802 and Karimnagar Makka-1 (−6.50%, P≤0.01), which evidently represent an easier standard for earliness. Two further crosses, DH6 × KML-137 and DH23 × KML-137, also reached significance against NK6802 and Karimnagar Makka-1 alone. The same pattern held for days to 50% silking and days to maturity, with DH13 × KML-137 significant against every check on both traits. Sharma *et al*. (2017) reported a broadly similar direction of heterosis but a much larger share of significant crosses, most likely because their single check flowered and matured later than the checks used here, making it an easier standard to beat an effect visible within the present study itself, where NK6802 and Karimnagar Makka-1 behaved as consistently easier standards for earliness than the other three checks.

Plant height and ear height exhibited significant and high positive mid-parent heterosis across all crosses, with values ranging from 26.92 to 61.89% for plant height and 30.71 to 74.80% for ear height (Table 1). Heterobeltiosis was also significant in the majority of crosses for both traits. However, none of the hybrids showed significant positive standard heterosis when compared with any of the five commercial checks. For plant height, standard heterosis varied from −19.87 to 4.42% over DHM-206, where 11 crosses were significantly shorter than the check, to −14.18 to 11.82% over Karimnagar Makka-1, with no significant differences observed in either direction. The most stringent comparison was against DHM-117, where standard heterosis ranged from −21.60 to 2.16%, and 16 crosses were significantly inferior for plant height. A similar trend was observed for ear height, with DHM-117 representing the most demanding check, whereas Karimnagar Makka provided the least stringent comparison. Although the hybrid DH22 × KML-137 recorded the highest standard heterosis over NK6802 and Karimnagar Makka-1, its superiority was not statistically significant. Similar findings were reported by Sharma *et al*. (2017), who noted that high levels of heterosis over the parents did not necessarily translate into superiority over commercial checks. The present multi-check evaluation further demonstrates that conclusions based on standard heterosis are influenced by the choice of check, as different commercial hybrids impose different levels of comparison.

A similar trend was observed for the four ear-related traits. Ear length and ear girth exhibited consistently high and significant mid-parent heterosis, ranging from 18.18 to 57.32% and 24.76 to 55.71%, respectively, whereas significant mid-parent heterosis for kernel rows per ear was observed in only 9 of the 40 crosses. For ear length, standard heterosis ranged from −25.84 to 2.07% over DHM-117, the most stringent check, where 22 crosses were significantly inferior, to −17.29 to 13.83% over Karimnagar Makka-1, the least stringent check. Only DH22 × KML-137 and DH23 × KML-87 recorded significant positive standard heterosis (13.83%; $P \leq 0.05$) over Karimnagar Makka-1. Ear girth showed a similar pattern, with no hybrid exhibiting significant positive standard heterosis against any check. In contrast, DH22 × KML-137, DH23 × KML-87 and DH23 × KML-137 recorded significant positive standard heterosis of 28.57% ($P \leq 0.01$) for kernel rows per ear, while DH22 × KML-137 and DH23 × KML-87 showed significant positive standard heterosis (16.90%; $P \leq 0.05$) for kernels per row, both only over Karimnagar Makka-1. These findings agree with Sharma *et al*. (2017), who also reported substantially higher heterosis over the parents than over the commercial check for ear-related traits, indicating that superiority over commercial hybrids is more difficult to achieve than superiority over the parental lines.

Test weight exhibited a similar pattern, with standard heterosis ranging from −23.16 to 5.65% over DHM-206, where 21 crosses were significantly lighter than the check and none were superior, to −16.56 to 14.72% over Karimnagar Makka-1, where 10 crosses were significantly lighter and three were significantly heavier, including DH22 × KML-137 and DH23 × KML-87 (14.72%). The most stringent comparison was against DHM-117, with 31 of the 40 crosses recording significantly lower test weight. In contrast, shelling percentage showed a wider numerical variation across checks, ranging from −9.07% over DHM-117 to 3.35% over Karimnagar Makka-1. However, none of the hybrids exhibited significant standard heterosis against any of the five checks, suggesting that the observed differences were not statistically meaningful. Moisture content displayed a distinct response, with significant and desirable negative standard heterosis observed only for DH9 × KML-87 and DH20 × KML-137 (−7.29%; $P \leq 0.05$) over DHM-206, while no significant differences were detected against the remaining four checks.

Grain yield, the primary trait of interest, exhibited significant and high mid-parent heterosis and heterobeltiosis in all 40 crosses, with the maximum values of 318.05% and 308.10%, respectively, recorded in DH23 × KML-137 (Table 1). In contrast, standard heterosis varied considerably depending on the commercial check used (Table 2). Against the two highest-yielding checks, DHM-206 (8423 kg ha^{-1}) and DHM-182 (8583 kg ha^{-1}), standard heterosis ranged from −51.65 to 8.04% and −52.55 to 6.03%, respectively. In both comparisons, 35 of the 40 crosses exhibited significant negative standard heterosis, and none significantly outperformed either check, although DH22 × KML-137 and DH23 × KML-137 recorded the highest values. A different trend was observed when compared with the relatively lower-yielding checks, NK6802 (7178 kg ha^{-1}), DHM117 (7833 kg ha^{-1}) and Karimnagar Makka-1 (7211 kg ha^{-1}), where standard heterosis increased to 26.78, 16.18 and 26.21%, respectively. Five hybrids (DH11 × KML-87, DH17 × KML-87, DH22 × KML-137, DH23 × KML-87 and DH23 × KML-137) consistently exhibited significant positive standard heterosis over all three checks, with DH22 × KML-137 recording the highest values. Against DHM-117, all crosses differed significantly from the check, with 35 showing negative and five showing positive standard heterosis, indicating that it served as the most discriminating check for grain yield.

These results are consistent with Sharma *et al*. (2017), who also observed high parental heterosis but comparatively limited standard heterosis for grain yield. However, the present study further demonstrates that the magnitude of standard heterosis is strongly influenced by the choice of commercial check. Similar levels of positive standard heterosis over commercial checks have also been reported by Beyene *et al*. (2011), who recorded values of up to 29.5%, and by Sserumaga *et al*. (2016), who found doubled haploid testcrosses exceeding commercial checks by as much as 49%. The relatively low standard heterosis observed against DHM-206 and DHM-182 is likely due to their superior yield potential as elite commercial hybrids, whereas the significant superiority of several hybrids over NK6802, DHM-117 and Karimnagar Makka-1 demonstrates their competitive performance against a substantial proportion of the commercial hybrids currently in cultivation. These findings highlight the importance of evaluating standard heterosis using multiple commercial checks, as conclusions based on a single reference hybrid may underestimate the commercial potential of promising genotypes.

Starch content exhibited a distinct pattern compared with the other traits. Although significant positive mid-parent heterosis was observed in 15 of the 40 crosses, reaching a maximum of 10.24% in DH8 × KML-137, none of the hybrids showed significant heterobeltiosis or significant standard heterosis against any of the five commercial checks. Among the hybrids, DH8 × KML-137 consistently recorded the highest positive standard heterosis across all five checks, ranging from 0.07% over DHM-182 to 1.46% over Karimnagar Makka-1, followed by DH19 × KML-87, although neither achieved statistical significance. Similar observations were reported by Sharma *et al*. (2017), while Bisen *et al*. (2017) and Khan *et al*. (2018) also found that starch and other quality traits exhibited greater heterosis over the parents than over commercial checks. The present multi-check evaluation further confirms that starch content is less responsive to heterosis at the commercial check level, suggesting that improvement of this trait may depend more on direct selection of superior parental lines than on exploiting hybrid vigour alone.

Across all traits, DH22 × KML-137 emerged as the most promising hybrid, exhibiting the highest or near-highest standard heterosis for plant height, ear height, ear length, ear girth, kernel rows per ear, kernels per row, test weight and grain yield wherever significant positive standard heterosis was observed. DH23 × KML-137 and DH23 × KML-87 also performed consistently well and, together with DH11 × KML-87 and DH17 × KML-87, formed the group of five hybrids that significantly outperformed three of the five commercial checks for grain yield. DH13 × KML-137 was identified as the best hybrid for earliness across all comparisons, while DH8 × KML-137 was the most promising for starch content despite the absence of significant standard heterosis. These hybrids represent valuable candidates for further evaluation and breeding, and the check-wise analysis provides a clear indication of the commercial checks they have already equalled or surpassed, as well as those against which further improvement is required.

Table 1 : Range of mid-parent heterosis and heterobeltiosis, and number of significant crosses, for fourteen characters in doubled haploid derived crosses of maize

Character	MP heterosis (%)	Heterobeltiosis (%)	Sig. crosses (MP)	Sig. crosses (BP)
Days to 50% anthesis	-7.26 to 0.00	-9.45 to -1.60	23	23
Days to 50% silking	-6.92 to 0.00	-9.02 to -1.53	23	23
Days to maturity	-3.07 to 0.88	-4.33 to 0.00	18	13
Plant height (cm)	26.92 to 61.89	19.17 to 58.19	40	38
Ear height (cm)	30.71 to 74.80	21.13 to 66.18	40	40
Ear length (cm)	18.18 to 57.32	8.06 to 52.51	40	29
Ear girth (cm)	24.76 to 55.71	14.10 to 49.32	40	38
Kernel rows per ear	-7.69 to 50.00	-14.29 to 50.00	9	3
Kernels per row	17.02 to 72.92	3.77 to 62.75	32	25
Test weight (g)	15.90 to 54.20	5.93 to 49.90	40	32
Starch content (%)	0.80 to 10.24	0.56 to 9.37	15	0
Moisture content (%)	3.15 to 11.66	1.78 to 11.66	20	7
Shelling percentage (%)	4.34 to 14.57	2.31 to 13.34	16	4
Grain yield (kg/ha)	80.67 to 318.05	69.13 to 308.10	40	40

MP = mid-parent heterosis; BP = heterobeltiosis. Sig. crosses = number of the 40 crosses significant at $P \leq 0.05$ or $P \leq 0.01$ (either direction). Ranges and significant-cross counts for mid-parent heterosis and heterobeltiosis are unaffected by the number of checks used, since neither is computed relative to a check.

Table 2 : Standard heterosis (%) for fourteen characters in doubled haploid derived crosses of maize, computed independently against each of five commercial hybrid checks

Character	DHM-206 (Check 1)	DHM-182 (Check 2)	NK6802 (Check 3)	DHM-117 (Check 4)	Karimnagar Makka-1 (Check 5)
Days to 50% anthesis	-4.96 to 1.65 (1)	-4.96 to 1.65 (1)	-6.50 to 0.00 (3)	-4.96 to 1.65 (1)	-6.50 to 0.00 (3)
Days to 50% silking	-4.72 to 1.57 (1)	-4.72 to 1.57 (1)	-6.20 to 0.00 (3)	-4.72 to 1.57 (1)	-6.20 to 0.00 (3)
Days to maturity	-2.64 to 1.76 (1)	-2.64 to 1.76 (1)	-3.49 to 0.87 (3)	-2.64 to 1.76 (1)	-3.49 to 0.87 (3)
Plant height (cm)	-19.87 to 4.42 (11)	-18.79 to 5.82 (9)	-16.17 to 9.24 (3)	-21.60 to 2.16 (16)	-14.18 to 11.82 (0)
Ear height (cm)	-23.85 to 3.67 (16)	-22.43 to 5.61 (16)	-19.42 to 9.71 (8)	-25.23 to 1.80 (20)	-17.82 to 11.88 (6)
Ear length (cm)	-23.06 to 5.90 (18)	-21.80 to 7.63 (16)	-20.06 to 10.03 (11)	-25.84 to 2.07 (22)	-17.29 to 13.83 (9)
Ear girth (cm)	-18.97 to 5.14 (15)	-17.65 to 6.86 (10)	-15.72 to 9.36 (6)	-21.00 to 2.51 (20)	-13.99 to 11.60 (2)
Kernel rows per ear	-25.00 to 12.50 (3)	-25.00 to 12.50 (3)	-25.00 to 12.50 (3)	-25.00 to 12.50 (3)	-14.29 to 28.57 (3)
Kernels per row	-31.17 to 7.79 (19)	-29.33 to 10.67 (16)	-27.40 to 13.70 (12)	-34.57 to 2.47 (26)	-25.35 to 16.90 (10)
Test weight (g)	-23.16 to 5.65 (21)	-22.29 to 6.86 (18)	-19.29 to 10.98 (16)	-25.89 to 1.91 (31)	-16.56 to 14.72 (10)
Starch content (%)	-7.79 to 1.17 (0)	-8.79 to 0.07 (0)	-8.06 to 0.87 (0)	-8.13 to 0.80 (0)	-7.52 to 1.46 (0)
Moisture content (%)	-7.29 to 0.81 (2)	-5.76 to 2.47 (0)	-4.18 to 4.18 (0)	-6.53 to 1.63 (0)	-2.55 to 5.96 (0)
Shelling percentage (%)	-8.31 to 1.55 (0)	-7.98 to 1.92 (0)	-7.59 to 2.35 (0)	-9.07 to 0.71 (0)	-6.69 to 3.35 (0)
Grain yield (kg/ha)	-51.65 to 8.04 (35)	-52.55 to 6.03 (35)	-43.26 to 26.78 (39)	-48.00 to 16.18 (40)	-43.52 to 26.21 (39)

Each cell gives the range of standard heterosis (%) over the named check across the 40 crosses, with the number of crosses significant at $P \leq 0.05$ or $P \leq 0.01$ (either direction) shown in parentheses. DHM-206, DHM-182, NK6802, DHM117 and Karimnagar Makka-1 are the five commercial hybrid checks (Check 1 to Check 5) described in section 2.

Conclusion

The doubled haploid lines used in this study combined strongly with both testers relative to their own parental values, producing hybrids with substantial positive heterosis over the mid parent and the better parent for grain yield and almost all of its component traits. Benchmarked against five commercial checks rather than one, this heterosis translated into a materially different picture depending on which check was used: standard heterosis for grain yield was negative in the great majority of crosses against the two highest-yielding checks, DHM-206 and DHM-182, but five hybrids DH11 × KML-87, DH17 × KML-87, DH22 × KML-137, DH23 × KML-87 and DH23 × KML-137 recorded significant positive standard heterosis, up to 26.78%, against the three lower-yielding checks, NK6802, DHM-117 and Karimnagar Makka-1. This gap between better-parent and standard heterosis, evident for nearly every character examined here, and its sensitivity to the particular check chosen, indicates first that some of the checks used in this trial DHM-206 and DHM-182 in particular are already well adapted, high-performing hybrids that will require stronger parental combinations or further cycles of selection to beat, and second that evaluating standard heterosis against a single check can materially overstate or understate the competitiveness of a breeding population. A multi-check comparison, as used here, gives a more complete and more reliable picture of where these DH-derived hybrids actually stand relative to the commercial hybrids currently available. Starch content behaved differently from the agronomic traits, showing limited heterosis on any basis of comparison against any of the five checks, which suggests that improving this quality trait in the programme may depend more on direct selection among the parental lines than on the exploitation of hybrid vigour. On the strength of the present results, the crosses DH22 × KML-137, DH23 × KML-137, DH11 × KML-87, DH17 × KML-87 and DH23 × KML-87 (for grain yield), DH13 × KML-137 (for earliness) and DH8 × KML-137 (for starch content) are recommended for multi-location and multi-season testing, evaluated against the full panel of commercial checks where possible, to confirm their yield stability, earliness and starch performance before any advancement to hybrid release.

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