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## HETEROSIS IN MAIZE (*ZEA MAYS* L.) F1 HALF-DIALLEL HYBRIDS EVALUATED UNDER LOW-NITROGEN STRESS CONDITIONS

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### SUMMARY

Maize is one of the major crops in Indonesia; however, its national production remains fluctuating. The development of high-yielding hybrid maize cultivars with tolerance to low nitrogen is a vital aim to boost the production. The following research aimed to study the heterotic effects in 10 maize F1 half-diallel hybrids, evaluated under two nitrogen levels, to identify the high-yielding hybrids. The experiment layout had a split-plot design with two factors. The main plots received the nitrogen (N) levels (N1: 50% and N2: 100%), while the 10 F1 hybrids, five parental genotypes, and two check genotypes (NASA-29 and Pertiwi-3) entailed placement in the subplots. Calculating the analysis of variance and heterotic effects occurred for growth, ear, kernel, yield, and flowering traits, followed by heatmap and principal component analyses (PCA). The analysis of variance revealed significant interactions of nitrogen levels and maize genotypes for several traits. Heterotic effects were evident across nitrogen levels for all traits. Heatmap and PCA assisted in identifying the heterosis patterns. Overall, the maize hybrid H<sub>9</sub> (P3 × P5) excelled all other hybrids for most assessed traits, indicating better adaptability to low-nitrogen environments for use in the next breeding program.

**Keywords:** Growth and yield traits, half-diallel hybrids, heatmap analyses, heterosis, maize, nitrogen stress tolerance, principal component analysis

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**Key findings:** The F1 hybrids revealed heterotic effects for studied traits in maize (*Z. mays* L.) (up to 21% in growth, 48% in ear, 66% in kernel, and -28% in flowering traits). Nitrogen-by-genotype interactions were considerable across all traits, indicating better adoption to low nitrogen stress. The F1 hybrid H<sub>9</sub> (P3 × P5) was superior for almost all traits for further breeding programs' use. The heatmap analysis and PCA were notably useful in identifying heterosis patterns and potential hybrids.

## INTRODUCTION

Maize (*Zea mays* L.) is one of Indonesia's major crops, following rice, and plays an important role as a staple food, livestock feed, and an industrial raw material (Magfiroh *et al.*, 2018). Despite being affected by various factors, e.g., climate, pests and diseases, and land conversion, national maize production has continued to increase at an annual rate of 3% (15.14 million tons in 2024) (Syahrudin *et al.*, 2020; Central Bureau of Statistics, 2025a). However, this growth remains insufficient to meet domestic needs and compels the country to continue importing from countries like Argentina, Brazil, and India (1.38 million tons in 2024, 11.69% rise) (Purnamasari *et al.*, 2020; Central Bureau of Statistics, 2025b). Thus, achieving maize self-sufficiency requires boosting national production through the development of high-yielding and climate-adapted maize cultivars (Syahrudin *et al.*, 2020).

Maize breeding in Indonesia has progressed over the decades, primarily through conventional methods. Mostly, the newly released cultivars are hybrids (80%–90%), while others are open-pollinated. Notable maize hybrids include Bima-1 and Bima-2, known for their highest grain yield, downy mildew resistance, and better adaptability to suboptimal conditions. Other cultivars adhered to specific environments, such as the shade-tolerant JHANA, drought-tolerant JHARING, and prolific NASA-29 (Syahrudin *et al.*, 2020). However, the development of low nitrogen-tolerance maize varieties is still lacking. Nitrogen fertilizer remains a limiting factor in maize production. Fertilizer scarcity is often due to distribution issues that can lead to nutrient deficiency, stunted growth, and yield reduction (Widowati *et al.*, 2011).

Several Indonesian researchers have initiated investigations on low-nitrogen stress tolerance in maize. Efendi *et al.* (2017) examined the general and specific combining abilities (GCA and SCA) of some maize hybrids and identified three hybrids that outperformed the check cultivars under low-nitrogen stress. Efendi *et al.* (2024) reported nitrogen stress significantly affected the maize's agronomic traits, with numerous hybrids identified as low-N-tolerant, high-yielding, and stable—making them suitable for cultivation under limited nitrogen conditions. However, other studies within this specific theme are still few, as most research has focused on tolerance to other stresses, such as drought, flooding, and diseases (Muis *et al.*, 2015; Azrai *et al.*, 2022).

Superior, low nitrogen-tolerant maize hybrids can be developed by exploiting heterosis, a phenomenon in which F1 hybrids outperform their inbred parental lines (Batte *et al.*, 2020). The increased vigor associated with heterosis widely refers to non-additive gene effects, including dominance, overdominance, and epistasis (Fiévet *et al.*, 2018). Heterosis, often evaluated together with comparisons against check cultivars, has long been applicable to select promising candidates for the development of new varieties (Hochholdinger and Baldauf, 2018; Liu *et al.*, 2020). In maize, the assessment of resulting F1 hybrids for heterosis can proceed in key traits, such as growth, ear, kernel, yield, and flowering under low-nitrogen stress. The best-performing hybrids can become the selected candidates for subsequent breeding stages (Kustanto and Hendrayana, 2023).

Heterosis studies in maize for low-nitrogen stress tolerance have transpired in several cases. Riache *et al.* (2023) evaluated F1 maize hybrids and observed heterotic effects based on physiological and morphological responses under water and low

nitrogen-stress conditions in Algeria. Similarly, Makumbi *et al.* (2011) also reported considerable heterotic effects in F1 diallel hybrids under drought and low-nitrogen stress. Both studies confirmed the observed hybrid vigor was due to non-additive gene effects, leading to more efficient nitrogen use and identifying promising candidates for novel hybrid cultivars (Makumbi *et al.*, 2011; Riache *et al.*, 2023).

In our context, five maize inbred lines had previously succeeded in their development. The general combining ability (GCA) of the parental genotypes and the specific combining ability (SCA) of the resulting F1 hybrids have also attained evaluation (Aulianta *et al.*, 2022). Therefore, the presented study aimed to assess the heterotic effects in half-diallel F1 hybrids derived from these inbred lines to identify the promising hybrids with considerable heterosis under low-nitrogen stress environments.

## MATERIALS AND METHODS

### Experimental design and procedure

The maize field experiment started at the Assessment Institute for Agricultural Technology in Mojokerto, Jawa Timur Province,

Indonesia (7.5084° S, 112.5324° E). The site lies within a tropical monsoon climate (Am), with temperatures of 22 °C–35 °C, precipitation at 1,400–3,000 mm year<sup>-1</sup>, and a dry-rainy seasonal pattern (the dry season is June–September). The land is flat (0%–3% slope), and the soil is alluvial with an effective depth of ~90 cm. The study followed a split-plot design with two factors and three replications. The main plots comprised nitrogen levels, i.e., N1 (50%) and N2 (100%), while the subplots included maize genotypes, comprising five parental lines, 10 F1 half-diallel hybrids, and two standard check cultivars (Table 1).

All the maize genotypes' cultivation employed the recommended production technology and practices (Aulianta *et al.*, 2022). The fertilization regime followed Ariyanto *et al.* (2024), which included 15 t ha<sup>-1</sup> of manure applied before sowing, 300 kg ha<sup>-1</sup> of TSP, 100 kg ha<sup>-1</sup> of KCl, and urea treated in two doses: 100 kg ha<sup>-1</sup> for the 50% nitrogen level (N1) and 200 kg ha<sup>-1</sup> for the 100% nitrogen level (N2). Maize plants entailed harvest at 90–100 days after sowing (DAS) during the milky stage, indicated by drying silks, full-sized kernels with a milk, light yellowish color, and fully developed cobs with tightly packed kernels.

**Table 1.** Details of maize parental lines and their F1 half-diallel hybrids used in this study.

No.	Parental Code	Pedigree	Base population		
1	P1	A 25-1-4-1-1-1-1-1-B-B	Cultivar Arjuna (A), selfed eight times, and bulked two times		
2	P2	B 40-5-1-6-1-1-1-1-B-B	Cultivar Bisma (B), selfed eight times, and bulked two times		
3	P3	H 36-1-7-1-1-1-1-1-B-B	Popular hybrid population (H), selfed eight times, and bulked two times		
4	P4	(A/H) 52-3-1-4-1-1-1-1-B-B	Arjuna × popular hybrid population, selfed eight times, and bulked two times		
5	P5	(B/H) 33-1-5-1-3-1-1-1-B-B	Bisma × popular hybrid population, selfed eight times, and bulked two times		
No.	Code	Crosses	No.	Code	Crosses
1	H <sub>1</sub>	P1 × P2	6	H <sub>6</sub>	P2 × P4
2	H <sub>2</sub>	P1 × P3	7	H <sub>7</sub>	P2 × P5
3	H <sub>3</sub>	P1 × P4	8	H <sub>8</sub>	P3 × P4
4	H <sub>4</sub>	P1 × P5	9	H <sub>9</sub>	P3 × P5
5	H <sub>5</sub>	P2 × P3	10	H <sub>10</sub>	P4 × P5

### Data recording, ANOVA, and heterosis analysis

Grouping of observations by the trait categories occurred. The growth traits included plant height (cm) and the number of leaves. The ear traits comprised ear length (cm), diameter (cm), weight (g), and the number of ears. Meanwhile, kernel yield components consisted of grain weight per ear (g), the number of kernels per ear, kernel rows per ear, 100-kernels weight (g), and grain yield per hectare ( $t\ ha^{-1}$ ). Finally, flowering traits were days to anthesis (days), days to silking (days), and anthesis-silking interval (days).

All the recorded data based on various parameters incurred analysis using analysis of variance (ANOVA) to evaluate the effects of main plots, subplots, and their interaction. Testing of F-statistics was at 90% ( $p < 0.10$ ) and 95% ( $p < 0.05$ ) significance levels. Results presentation was by mean squares along with their significance levels. Heterosis calculation occurred by comparing the F1 hybrids' performance against their parental average (mid-parent heterosis: MP), the best parent (best-parent heterosis: BP), and the check cultivars (standard heterosis: SH), following the formulas described by Shrestha *et al.* (2018) and Geng *et al.* (2021). Defining the desired directions of heterosis were as follows: moderate positive heterosis for growth components, high positive for ear, kernel, and yield components, and negative heterotic effects for the maize flowering traits.

### Heatmap and PCA analyses

A generated heatmap was successful from standardized (Z-score) heterosis data from all traits to provide a heterosis pattern among hybrids. The data further sustained exploratory analysis using the PCA, calculated based on the correlation matrix, with two principal components extracted and analyzed. K-means clustering (with kmax determined using the Silhouette method) ensued, with the results visualized as score and loading plots. Both the conduct of heatmap and PCA analysis used R Studio (version 2025.11.0 build 217, R version

4.5.1) and ggplot2 packages (version 4.0.0) for plotting.

## RESULTS AND DISCUSSION

The analysis of variance revealed significant interaction effects between nitrogen levels and maize genotypes ( $N \times G$ ) for several traits, including plant height, ear weight, grain weight per ear, grain yield, days to anthesis, days to silking, and the anthesis-silking interval (Table 2). The interaction effects in these traits displayed the genotype-specific nitrogen use efficiency. In this study, the moderate 50% nitrogen reduction sought to evaluate the genotype effects of the hybrids under low-nitrogen stress while minimizing the risk of crop failure.

Nitrogen is a critical nutrient for maize and other plants, serving as a building block for key biomolecules, such as proteins, enzymes, and chlorophyll (Gheith *et al.*, 2022; Zayed *et al.*, 2023). Han *et al.* (2015) reported that under low-nitrogen stress, plants tend to prioritize nitrogen use efficiency over nitrogen uptake efficiency. The observed interaction supports the idea that some hybrids can utilize nitrogen more efficiently under nitrogen-limited conditions. In contrast, particular traits showed nonsignificant interaction effects, suggesting these traits were either more genetically stable and were less responsive to nitrogen application variations (Ivić *et al.*, 2021).

Heterotic effects of the hybrids were evident in all traits studied (Table 3 and Figure 1). Some F1 hybrids exhibited remarkable heterosis even under low nitrogen stress (50% dose), particularly for traits such as ear length (48.84% MP, 43.39% BP) and 100-kernel weight (66.22% MP, 63.71% BP). These results highlighted the hybrid vigor in F1 hybrids with superior adaptability and productivity under low nitrogen stress. The heterosis phenomenon widely refers to non-additive gene effects, such as dominance and epistasis, and has a considerable association with SCA than GCA (Fiévet *et al.*, 2018; Yu *et al.*, 2020). For hybridization, the proper selection of parental genotypes, along with

**Table 2.** Analysis of variance for growth, ear, kernel, yield, and flowering traits of maize genotypes evaluated under two nitrogen fertilization levels (50% and 100%).

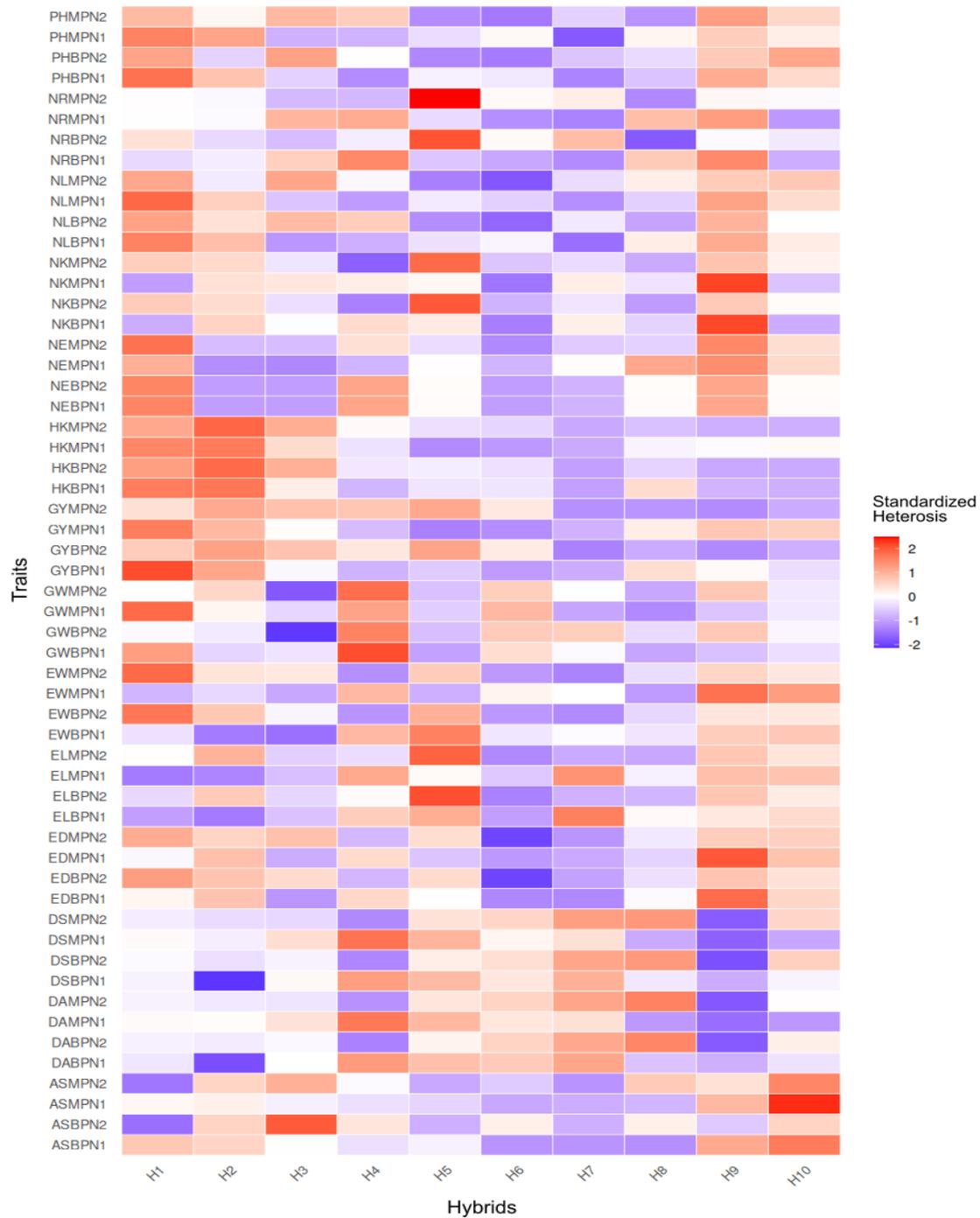
Source of variation	d.f.	Growth		Ear				Kernel and yield				Flowering			
		PH	NL	EL	ED	EW	NE	GWE	NKE	NKRE	HKW	GY	DA	DS	ASI
Replications	2	139 <sup>ns</sup>	2 <sup>ns</sup>	1.85 <sup>ns</sup>	17.45 <sup>ns</sup>	2295 <sup>ns</sup>	0.05 <sup>ns</sup>	1959 <sup>ns</sup>	968 <sup>ns</sup>	0.40 <sup>ns</sup>	82 <sup>ns</sup>	3.23 <sup>ns</sup>	8.9 <sup>ns</sup>	4.11 <sup>ns</sup>	0.91 <sup>ns</sup>
Nitrogen levels (N)	1	824412 <sup>ns</sup>	4840 <sup>ns</sup>	33.23 <sup>ns</sup>	0.01 <sup>ns</sup>	6806 <sup>ns</sup>	0.00 <sup>ns</sup>	15644 <sup>ns</sup>	60932 <sup>ns</sup>	9.83 <sup>ns</sup>	16 <sup>ns</sup>	11.81 <sup>ns</sup>	9.22 <sup>ns</sup>	5.49 <sup>ns</sup>	0.00 <sup>ns</sup>
Error (a)	4	210799	1216	28.00	32.89	8631	0.17	10699	47484	12.68	962	26.24	18.22	8.69	2.89
Genotypes (G)	16	1665 <sup>ns</sup>	3 <sup>ns</sup>	21.91 <sup>ns</sup>	35.68 <sup>ns</sup>	3514 <sup>ns</sup>	0.20 <sup>ns</sup>	1835 <sup>ns</sup>	10842 <sup>ns</sup>	4.81 <sup>ns</sup>	238 <sup>ns</sup>	7.38 <sup>ns</sup>	28.72 <sup>ns</sup>	34.53 <sup>ns</sup>	0.53 <sup>ns</sup>
N x G	16	56842**	305 <sup>ns</sup>	56.51 <sup>ns</sup>	13.95 <sup>ns</sup>	10019*	0.28*	6198*	35772 <sup>ns</sup>	7.49 <sup>ns</sup>	516 <sup>ns</sup>	25.93**	70.32**	81.56**	1.89 <sup>ns</sup>
Error (b)	64	16369	232	39.14	38.43	4794	0.43	2802	28387	9.83	329	11.25	27.54	29.44	1.18
Total	103	897	2	10.76	15.23	2076	0.18	927	7981	3.62	132	4.77	12.03	12.62	0.56

\*\*, \*: Significant at  $p \leq 0.01$  and  $p \leq 0.05$ , respectively, ns = Nonsignificant, PH = plant height, NL = number of leaves, EL = ear length, ED = ear diameter, EW = ear weight, NE = number of ears, GWE = grain weight ear<sup>-1</sup>, NKE = kernels ear<sup>-1</sup>, NKRE = kernel rows ear<sup>-1</sup>, HKW = 100-kernels weight, GY = grain yield ha<sup>-1</sup>, DA = days to anthesis, DS = days to silking, and ASI = anthesis-silking interval.

**Table 3.** Heterosis (%) ranges among the 10 maize F1 half-diallel hybrids evaluated under two-nitrogen fertilization levels (50% and 100%).

Traits	50% Nitrogen				100% Nitrogen			
	MP	BP	SH NASA-29	SH Pertiwi-3	MP	BP	SH NASA-29	SH Pertiwi-3
Growth traits								
Plant height	-22.31 – 21.90	-24.88 – 18.00	-26.23 – 9.32	-27.96 – 6.75	-25.90 – 23.71	-38.39 – 22.45	-26.35 – 30.45	-38.24 – 9.39
Number of leaves	-6.83 – 15.92	-9.64 – 15.19	-8.54 – 12.20	-12.79 – 6.98	-22.99 – 8.72	-22.55 – 3.92	-29.41 – 3.92	-26.53 – 8.16
Ear traits								
Ear length	-8.84 – 48.84	-15.97 – 43.39	-31.39 – 4.19	-31.03 – 4.74	-2.66 – 44.76	-15.04 – 33.11	-17.93 – 22.28	-29.00 – 5.79
Ear diameter	-7.95 – 12.17	-9.27 – 6.72	-0.13 – 23.28	-13.76 – 6.45	-26.23 – 9.87	-27.65 – 7.70	-23.17 – 9.95	-26.26 – 5.54
Ear weight	-13.73 – 41.27	-26.92 – 11.07	-16.91 – 51.60	-32.25 – 23.62	-43.86 – 69.98	-52.61 – 68.98	-47.12 – 32.06	-53.46 – 16.24
Number of ears	-37.93 – 30.43	-40.00 – 7.14	-10.00 – 50.00	-40.00 – 0.00	-33.33 – 36.36	-47.06 – 25.00	-35.71 – 7.14	-30.77 – 15.38
Kernel and yield traits								
Grain weight ear <sup>-1</sup>	-19.86 – 44.42	-35.45 – 26.00	-36.20 – 15.22	-36.64 – 14.42	-27.72 – 93.20	-29.35 – 66.79	-28.25 – 24.60	-26.26 – 28.06
Kernels ear <sup>-1</sup>	-14.54 – 30.13	-18.71 – 27.69	-26.11 – 3.24	-25.30 – 4.37	-40.48 – 58.10	-40.73 – 55.33	-44.99 – 24.66	-50.46 – 12.26
Kernel rows ear <sup>-1</sup>	-9.95 – 25.12	-10.38 – 20.95	-12.84 – 16.51	-20.83 – 5.83	-6.34 – 49.22	-18.64 – 35.85	-8.57 – 37.14	-25.58 – 11.63
100-kernels weight	-0.14 – 66.22	-16.11 – 63.71	-7.20 – 43.91	-1.66 – 52.50	-9.65 – 111.26	-23.75 – 98.04	-11.00 – 73.58	-11.50 – 72.59
Grain yield ha <sup>-1</sup>	-26.01 – 22.64	-30.02 – 16.94	-19.04 – 35.03	-16.91 – 38.57	8.77 – 126.50	-5.43 – 119.68	-23.13 – 46.63	-30.77 – 32.06
Flowering traits								
Days to anthesis	-1.15 – 13.13	-2.52 – 12.78	-0.79 – 8.48	6.34 – 16.28	-8.72 – 12.10	-9.94 – 11.00	-8.54 – 10.10	-5.23 – 14.08
Days to silking	-3.30 – 14.11	-4.35 – 13.77	-0.95 – 9.71	6.34 – 17.79	-8.29 – 10.16	-8.72 – 8.61	-6.82 – 10.80	-4.65 – 13.37
Anthesis-silking interval	-26.83 – 43.59	-28.57 – 33.33	-58.21 – (-16.42)	-53.33 – (-6.67)	-16.98 – 14.29	-24.00 – 5.26	-5.00 – 25.00	-5.00 – 25.00

MP = Mid-parent heterosis, BP = Best-parent heterosis or heterobelitosis, SH = Standard heterosis vs. checks (NASA 29 and Pertiwi 3).



**Figure 1.** Heatmap showing the standardized heterosis patterns (Z scores) of 10 maize hybrids (H<sub>1</sub>–H<sub>10</sub>) across growth, ear, kernel, yield, and flowering traits. Red color indicates positive, while blue color indicates negative heterosis. The relative magnitude of heterosis appears by color intensity. H<sub>1</sub> to H<sub>10</sub> = F1 diallel hybrids, PH = plant height, NL = number of leaves, EL = ear length, ED = ear diameter, EW = ear weight, NE = number of ears, GW = grain weight ear<sup>-1</sup>, NK = kernels ear<sup>-1</sup>, NR = kernel rows ear<sup>-1</sup>, HK = 100-kernels weight, GY = grain yield ha<sup>-1</sup>, DA = days to anthesis, DS = days to silking, AS = anthesis-silking interval, BP = best-parent heterosis, MP = mid-parent heterosis, N1 = 50% nitrogen, N2 = 100% nitrogen.

heterosis analysis of the resulting F1 hybrids, emerged as the critical steps in identifying the candidates for superior cultivars (Xing *et al.*, 2014).

For growth components (Figure 1), the F1 hybrids H<sub>1</sub> (P1 × P2) and H<sub>9</sub> (P3 × P5) appeared as the most desirable hybrids, showing moderate performance in plant height and the number of leaves in maize. Heterotic effects in growth traits, such as plant height and leaf number, were particularly important under low-nitrogen stress, as they promote early vigor, enhance nitrogen uptake efficiency, and support greater biomass accumulation. This early advantage contributes to stress resilience and yield stability, making growth-related heterosis a valuable trait for hybrid maize breeding under low-nutrient stress environments (Adu *et al.*, 2022; Santos *et al.*, 2023).

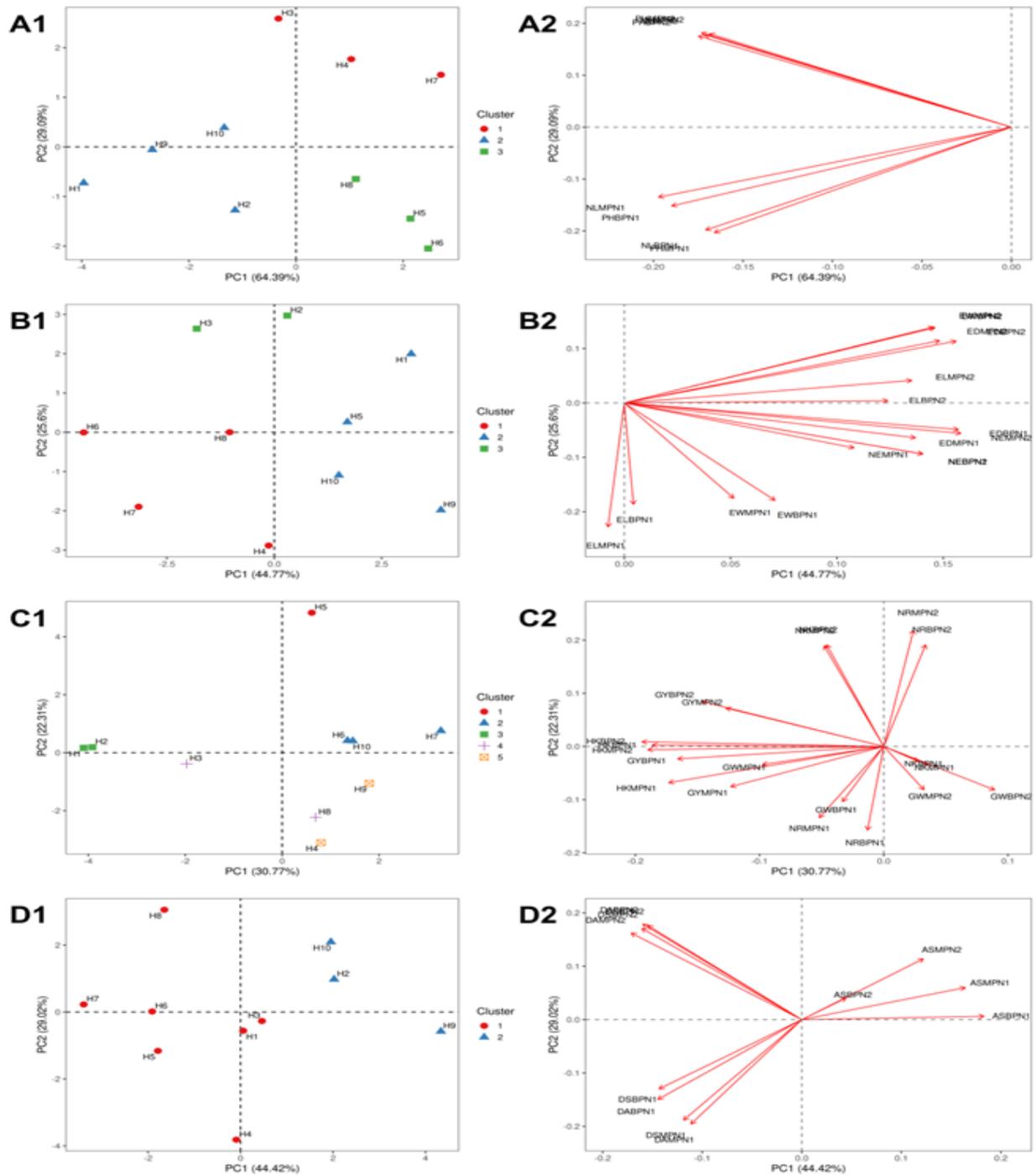
For ear components (Figure 1), the maize F1 hybrids H<sub>9</sub> (P3 × P5) and H<sub>10</sub> (P4 × P5) were the most promising, indicated by a positive heterosis pattern across nearly all parameters. The F1 hybrids with larger ear dimensions were the general preference of breeders, as they tend to have more kernel rows, higher kernel density, and greater grain yields (Yuwono *et al.*, 2017). For kernel and yield traits (Figure 1), the F1 hybrid H<sub>1</sub> (P1 × P2), H<sub>2</sub> (P1 × P3), and H<sub>9</sub> (P3 × P5) stood out as the most desirable hybrids, particularly in grain weight ear<sup>-1</sup>, kernels ear<sup>-1</sup>, kernel rows ear<sup>-1</sup>, and grain yield hectare<sup>-1</sup>. The kernel and yield traits were often the primary targets in breeding, especially under low-nitrogen stress, as these traits proved to have direct links to overall yield potential and productivity (Xiao *et al.*, 2021). In these traits, the observed heterosis reflects more efficient nitrogen utilization and effective translocation, which support improved biomass accumulation and grain yield under low-nitrogen stress (Han *et al.*, 2015; Riache *et al.*, 2023).

For flowering components (Figure 1), the F1 hybrids H<sub>1</sub> (P1 × P2) and H<sub>2</sub> (P1 × P3) exhibited negative heterosis across most parameters. Notably, the F1 hybrid H<sub>9</sub> (P3 × P5) also showed considerable negative heterotic effects for all the flowering traits,

except the anthesis-silking interval (ASI). In flowering traits, negative heterosis is often desirable as it indicates early maturity, shorter growth duration, and reduced time to harvest (Yuwono *et al.*, 2017). Under low nitrogen stress, flowering stages, such as anthesis and silking, become typically delayed, usually resulting in an extended anthesis-silking interval (Begizew and Desalegn, 2019). However, a prolonged ASI can disrupt the synchrony between pollen shedding and silk emergence, leading to protandrous flowering, exposed ear tips, and even the occurrence of ear barrenness (Edmeades *et al.*, 2000).

The PCA results in Figure 2 provide meaningful insight into the heterosis pattern. For the growth traits, all variables expressed positive correlations and contributed similarly to PC2. However, on PC1, the traits associated with 100% and 50% nitrogen showed opposite directions, indicating a clear effect of nitrogen levels on growth-related heterosis. Cluster 2 (H<sub>1</sub>, H<sub>2</sub>, H<sub>9</sub>, and H<sub>10</sub>) represented the desirable hybrids because its position was in the same direction as the contributing traits along PC1. For the ear traits, most variables demonstrated positive correlations and contributed strongly to PC1, except for ear length and ear weight, which unexpectedly showed higher loadings on PC2. Cluster 2 (H<sub>1</sub>, H<sub>5</sub>, H<sub>9</sub>, and H<sub>10</sub>) was distinctly the desirable group since it aligned with the direction of most traits in the loading plot.

For the kernel and yield traits, both the direction and magnitude of the loadings varied substantially across the two principal components, indicating a complex and diverse performance of the hybrids for these traits. Several variables contributed strongly to the overall variance, including 100-kernels weight and grain yield hectare<sup>-1</sup> on PC1, as well as kernels ear<sup>-1</sup> and kernel rows ear<sup>-1</sup> on PC2. The score plot revealed the highest level of cluster separation, with nine distinct groups, highlighting the heterogeneous nature of kernel and yield heterosis. Cluster 3 (H<sub>1</sub> and H<sub>2</sub>) prevailed as the desirable group, particularly for traits such as 100-kernel weight, grain yield hectare<sup>-1</sup>, and grain weight ear<sup>-1</sup>.



**Figure 2.** Score and loading plots generated from the PCA of heterosis among 10 maize F1 half-diallel hybrids evaluated under two nitrogen levels for growth (A1–A2), ear (B1–B2), kernel and yield (C1–C3), and flowering (D1–D3) traits. Exclusion of standard heterosis occurred from the PCA of flowering components due to multicollinearity. H<sub>1</sub> to H<sub>10</sub> = F1 diallel hybrid codes, PH = plant height, NL = number of leaves, EL = ear length, ED = ear diameter, EW = ear weight, NE = number of ears, GW = grain weight ear<sup>-1</sup>, NK = kernels ear<sup>-1</sup>, NR = kernel rows ear<sup>-1</sup>, HK = 100-kernels weight, GY = grain yield ha<sup>-1</sup>, DA = days to anthesis, DS = days to silking, AS = anthesis-silking interval, BP = best-parent heterosis, MP = mid-parent heterosis, N1 = 50% nitrogen, N2 = 100% nitrogen.

For the flowering traits, ASI projected in the opposite direction from days to anthesis and days to silking on PC1, supporting earlier findings that the hybrids tended to exhibit shorter anthesis and silking periods but a longer ASI. On PC2, days to anthesis and days to silking under the 100% and 50% nitrogen levels had orientations in the opposite direction, indicating a clear influence of nitrogen availability on these traits. The score plot indicated two distinct clusters, with Cluster 2 (H<sub>2</sub>, H<sub>9</sub>, and H<sub>10</sub>) noted for shorter days to anthesis and days to silking, but longer ASI.

Heterosis studies often involve large datasets, making it challenging to identify the general patterns among the F1 hybrids and define the heterotic groups. As an exploratory tool, PCA helps reduce data complexity into a few principal components (two in this study), allowing obvious distinction of high-performing hybrids under specific conditions (Al-Naggar *et al.*, 2022). Kumar *et al.*'s (2017) findings also demonstrated that hybrid clustering based on PCA score plots accurately reflects heterotic relationships and aids in the selection. These past findings supported our integrated approach combining ANOVA, heterosis calculation, heatmap, and PCA analyses to explore and validate the heterosis patterns and identify the elite maize hybrid candidates for further improvement in maize production.

## CONCLUSIONS

This study successfully evaluated the F1 half-diallel maize hybrids based on their heterotic effects in growth, ear, kernel, yield, and flowering traits under low-nitrogen stress. Analysis of variance revealed significant interaction effects between nitrogen doses and genotypes for several traits, indicating a genetic response to low nitrogen stress. The F1 hybrid H<sub>9</sub> (P3 × P5) emerged as the most consistently performing hybrid across all components, while H<sub>1</sub>, H<sub>2</sub>, and H<sub>10</sub> were also notable hybrids in particular components. The PCA analyses proved helpful in managing the complexity of the dataset and identifying the promising hybrids. Overall, the research results provided a solid base for further studies, such

as the DUST test, agronomic test, and multi-location trials, targeting the best-performing hybrids, aimed at producing novel, superior, and high-yielding maize cultivars well adapted to low nitrogen stress.

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