



## Cross Direction Determines Maternal Effects and Seed Morphometric Heterosis in Common Bean (*Phaseolus vulgaris* L.) Under Semi-Arid Conditions

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### Abstract

Hybrid vigor in common bean is often trait-specific and strongly shaped by reciprocal and maternal effects. This study evaluated reciprocal F1 crosses between Kakunzu, a drought-tolerant landrace, and Nyota, an iron-biofortified Kenyan cultivar, under semi-arid conditions in Machakos County, Kenya. Crosses were produced in both directions and assessed with their parents in a randomized complete block design. Kakunzu × Nyota showed superior seed performance, with the highest average seed weight (1.96 g), seed width (47.02), and projected area (2800), outperforming Nyota, Kakunzu, and Nyota × Kakunzu. Heterosis was asymmetric: Kakunzu × Nyota recorded positive mid-parent heterosis of 10.84% for width, 11.46% for area, and 26.05% for weight, plus positive heterobeltiosis, while the reciprocal cross showed negative heterosis. Image-based phenotyping and seed-level replicates confirmed maternal inheritance of seed-coat appearance. These findings show that cross direction determines seed-trait heterosis and support Kakunzu as the maternal parent for bean improvement in drought-prone environments.

**Keywords:** Biofortifications; Reciprocal Crosses; Seed Coat Phenotype; Semi-Arid Breeding; Food Security

### Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the world's most important grain legumes for direct human consumption and is a major source of dietary protein, minerals, and income in eastern and southern Africa [1,2]. In dryland production systems, however, bean productivity remains constrained by erratic rainfall, recurrent drought, poor soil fertility, and high seasonal variability, all of which reduce stand establishment and yield stability [2,3].

A practical breeding strategy for such environments is to combine the local adaptation of farmer-maintained landraces with

the agronomic or nutritional advantages of improved cultivars. Landraces can contribute to drought adaptation, resilience under low-input management, and local market acceptance, whereas improved cultivars may contribute to higher yield potential and enhanced nutritional quality. In common bean, iron biofortification has become an important breeding target because bean grain is a staple food for populations at risk of iron deficiency, and the crop has proven amenable to genetic improvement for higher seed iron concentration [4].

Hybrid vigor, or heterosis, refers to the superior performance of hybrid offspring relative to their parents for one or more

traits [5]. Although heterosis is less routinely exploited in self-pollinating crops than in cross-pollinated species, it can still be agronomically useful when the parental combination and target trait are appropriate. In seed crops, reciprocal effects are especially important because seed development is influenced by maternal tissues. The seed coat is maternally derived, and its development can regulate nutrient transfer, physical protection, and final seed phenotype [6]. In common bean specifically, reciprocal F1 hybrids can differ substantially in seed size and early seedling performance when maternal effects are strong [7].

The present study examined reciprocal F1 crosses between Kakunzu, a locally adapted drought-tolerant landrace, and Nyota, an iron-biofortified cultivar used in Kenya. The objective was to determine whether crossing direction influenced seed morphometric traits and seed-coat phenotype under semi-arid field conditions. We hypothesized that the maternal parent would strongly condition seed appearance and that heterosis for seed size would be expressed asymmetrically between reciprocal crosses.

## Materials and Methods

### Study site and plant materials

The experiment was conducted in Machakos County, Kenya, a semi-arid agro-ecological zone characterized by bimodal rainfall, frequent intra-seasonal moisture stress, and predominantly sandy-loam soils. Two contrasting bean genotypes were used as parents: Kakunzu, a farmer-maintained landrace valued locally for adaptation to low-rainfall environments, and Nyota, an improved iron-biofortified cultivar. These parents were selected to test whether reciprocal crossings could combine local adaptation with improved grain quality.

### Crossing scheme and field evaluation

Controlled hand pollinations were performed to generate the reciprocal F1 combinations Kakunzu × Nyota and Nyota × Kakunzu. The parental lines and both reciprocal hybrids were evaluated in the subsequent season using a randomized complete block design with three replicates. Plots consisted of four rows approximately 3 m long, with 0.5 m spacing between rows and about 10 cm between plants. Standard agronomic practices were applied uniformly, and the crop depended on seasonal rainfall without supplemental irrigation.

### Trait measurement

Phenological observations included emergence, flowering, and maturity. The present manuscript, however, focused on seed-related traits. After harvest, representative seed photographs were taken and seed morphometric traits were summarized as length, width, projected area, and seed weight. Seed-coat phenotype was documented visually and quantitatively. The image-based component complemented, rather than replaced, field observations.

### Image-based seed-coat phenotyping

To quantify reciprocal differences in seed-coat phenotype, representative seed images were used (Figure 1) and analyzed using a semi-automated image-processing workflow. Seed regions were segmented from the background by color-distance thresholding in CIELAB space, followed by morphological cleaning to obtain binary masks. Within each segmented seed region, mean lightness ( $L^*$ ),  $a^*$ , and  $b^*$  were computed. Pixels were further classified into maroon and cream hue classes, allowing calculation of maroon fraction and cream fraction for each genotype. To assess maternal resemblance, each hybrid was compared with both parents in standardized feature space using  $L^*$  and maroon fraction, and the hybrid was assigned to the nearest parental centroid.

### Seed-level technical replicate analysis

For a more reproducible image-analysis result, the representative seed panel was decomposed into 12 seed-level observations (three seeds per genotype). For each seed, CIELAB lightness, chromatic coordinates, maroon fraction, cream fraction, and segmented seed area were measured. Because these replicate observations were derived from the representative figure rather than from the full set of biological replicate images, they are treated as technical image replicates and interpreted accordingly.

### Statistical analysis and heterosis calculations

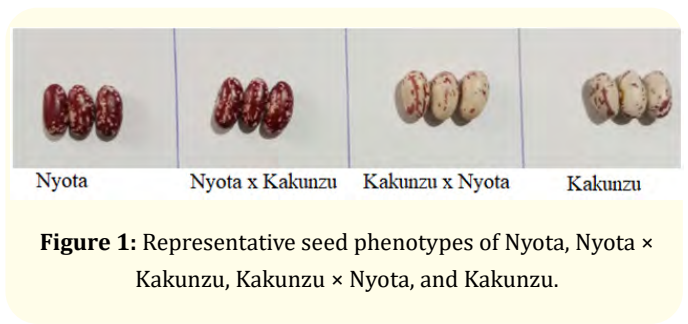
Differences among genotypes for quantitative traits were assessed in the original study using analysis of variance at  $\alpha = .05$ . For the seed-coat image analysis, one-way ANOVA and pairwise comparisons were applied to the seed-level technical replicate dataset. Heterosis metrics were calculated from genotype means using the following equations: mid-parent value (MP) =  $(P1 + P2) / 2$ ; mid-parent heterosis (MPH, %) =  $[(F1 - MP) / MP] \times 100$ ; better-parent heterosis or heterobeltiosis (BPH, %) =  $[(F1 - BP) / BP] \times 100$ , where BP is the better parent; reciprocal effect (RE) =

$F1[Kakunzu \times Nyota] - F1[Nyota \times Kakunzu]$ ; reciprocal effect (%) =  $[RE / \text{mean of the two reciprocal } F1s] \times 100$ ; and maternal effect estimate (MEE, %) =  $[(F1 - \text{maternal parent}) / \text{maternal parent}] \times 100$ .

## Results

### Reciprocal differences in seed phenotype

The reciprocal hybrids differed clearly in seed appearance. Nyota and Nyota  $\times$  Kakunzu showed the dark maroon-with-flecks phenotype, while Kakunzu and Kakunzu  $\times$  Nyota showed a lighter cream background with maroon speckling. This qualitative pattern is consistent with maternal inheritance of the seed coat, because each F1 resembled its female parent rather than its pollen parent. The reciprocal hybrids visually matched the maternal parent seed-coat phenotype, supporting strong maternal control of seed appearance.



**Figure 1:** Representative seed phenotypes of Nyota, Nyota  $\times$  Kakunzu, Kakunzu  $\times$  Nyota, and Kakunzu.

### Seed morphometric variation among parents and reciprocal hybrids

Seed morphometric measurements showed that the reciprocal hybrid Kakunzu  $\times$  Nyota had the broadest seeds, the largest projected seed area, and the highest 4-seed weight. By contrast, Nyota  $\times$  Kakunzu remained similar to the parents for these traits. Seed length varied less strongly than the other seed dimensions, indicating that cross direction primarily affected width, area, and mass rather than elongation.

Genotype	Length	Width	Area	Average seed weight (g)
Nyota	79.72 $\pm$ 1.52	40.05 $\pm$ 3.12	2468 $\pm$ 176	1.56
Nyota $\times$ Kakunzu	80.88 $\pm$ 4.70	39.36 $\pm$ 5.43	2410 $\pm$ 419	1.52
Kakunzu $\times$ Nyota	77.29 $\pm$ 4.93	47.02 $\pm$ 3.09	2800 $\pm$ 340	1.96
Kakunzu	73.70 $\pm$ 2.66	44.79 $\pm$ 9.36	2556 $\pm$ 600	1.55

**Table 1:** Seed morphometric traits of parental lines and reciprocal F1 hybrids.

Values are reported as genotype means with their accompanying variability term from the study dataset.

### Heterosis, reciprocal effects, and maternal effects

Heterosis analysis showed a strongly asymmetric response between reciprocal hybrids. Kakunzu  $\times$  Nyota expressed positive mid-parent heterosis and heterobeltiosis for seed width, projected area, and 4-seed weight, whereas Nyota  $\times$  Kakunzu showed negative heterosis for the same traits. The largest heterotic

response occurred for 4-seed weight, where Kakunzu  $\times$  Nyota achieved 26.05% mid-parent heterosis and 25.64% heterobeltiosis. Reciprocal effects also favored Kakunzu as the maternal parent for width, area, and weight. These results quantitatively confirm that the direction of crossing was critical for realizing favorable seed-size heterosis.

Trait	MPH (%) N $\times$ K	MPH (%) K $\times$ N	BPH (%) N $\times$ K	BPH (%) K $\times$ N	RE	RE (%)	MEE (%) N $\times$ K	MEE (%) K $\times$ N
Length	5.44	0.76	1.46	-3.05	-3.59	-4.54	1.46	4.87
Width	-7.21	10.84	-12.12	4.98	7.66	17.74	-1.72	4.98
Area	-4.06	11.46	-5.71	9.55	390.00	14.97	-2.35	9.55
Weight	-2.25	26.05	-2.56	25.64	0.44	25.29	-2.56	26.45

**Table 2:** Mid-parent heterosis (MPH), heterobeltiosis (BPH), reciprocal effects (RE), and maternal effect estimates (MEE) for seed traits.

**Quantitative image analysis confirms maternal inheritance of seed-coat phenotype**

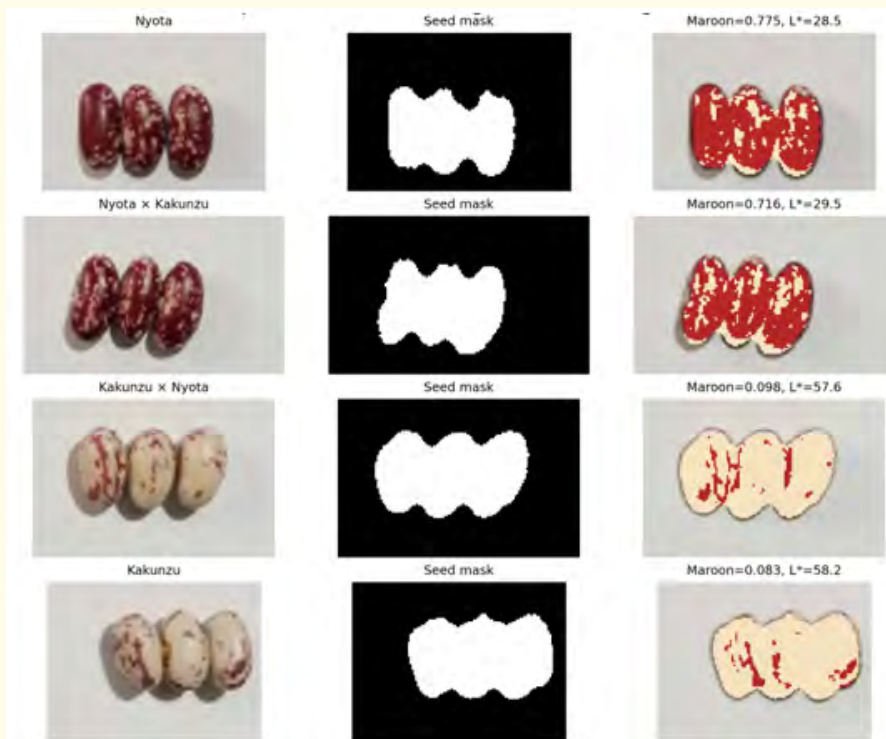
Image-based seed-coat analysis clearly separated the maroon Nyota-type seeds from the cream-speckled Kakunzu-type seeds. Nyota and Nyota × Kakunzu had low lightness and high maroon fraction, whereas Kakunzu and Kakunzu × Nyota had high lightness and low maroon fraction. In standardized feature space, each

reciprocal F1 was classified as most similar to its maternal parent. Seed-level technical replicate analysis further showed significant genotype effects for lightness, chromatic coordinates, and maroon fraction, while both reciprocal hybrids remained statistically indistinguishable from their respective maternal parents for the major seed-coat color traits (Figure 2).

Genotype	L* (mean ± SE)	Maroon fraction (mean ± SE)	Maternal-like classification	Notes
Nyota	33.66 ± 0.22	0.724 ± 0.021	Parent reference	Dark maroon parent
Nyota × Kakunzu	34.34 ± 1.76	0.679 ± 0.027	Nyota-like	Not different from maternal parent for main color traits
Kakunzu × Nyota	58.46 ± 1.28	0.123 ± 0.043	Kakunzu-like	Not different from maternal parent for main color traits
Kakunzu	58.66 ± 1.47	0.090 ± 0.022	Parent reference	Cream-speckled parent

**Table 3:** Seed-level image-derived statistics for seed-coat phenotype (n = 3 seeds per genotype from the representative figure).

ANOVA summary: genotype effects were significant for L\* (F = 115.92, p = 6.24 × 10<sup>-7</sup>), a\* (F = 87.10, p = 2.00 × 10<sup>-6</sup>), b\* (F = 14.03, p = .0015), and maroon fraction (F = 135.32, p = 3.41 × 10<sup>-7</sup>).



**Figure 2:** Quantitative segmentation of representative seed coats into maroon and cream classes.

The reciprocal hybrids aligned with different parental color profiles, providing reproducible image-based support for maternal inheritance of seed-coat phenotype.

## Discussion

This study shows that reciprocal crossing direction strongly conditioned the expression of heterosis in common bean seed traits. The most important quantitative result was the superiority of Kakunzu × Nyota for seed width, projected area, and 4-seed weight. Because the reciprocal hybrid did not express the same advantage, the observed response cannot be explained by nuclear complementation alone. Instead, the results point to a strong maternal or reciprocal component that influenced seed development in the F1 generation. This interpretation is consistent with broader theory on heterosis as a context-dependent phenomenon shaped by dominance, overdominance, epistasis, and the biological setting in which traits are expressed [5].

The seed-coat data further supports maternal interpretation. Seed coat is derived from maternal tissue, and maternal tissues can regulate both the phenotype and developmental environment of the seed [6]. The visual evidence, the quantitative image analysis, and the seed-level technical replicate statistics all converged on the same pattern: Nyota × Kakunzu was Nyota-like, whereas Kakunzu × Nyota was Kakunzu-like. This result agrees with prior work in common bean showing maternal effects on seed size and early seedling traits in reciprocal hybrids [7].

The greater seed mass of Kakunzu × Nyota is agronomically relevant because seed size is often associated with seed vigor, rapid establishment, and early heterotrophic growth, particularly when seedlings experience stress during emergence [8,9]. In the present study, however, improved seedling establishment or drought resilience was not measured directly in enough detail to claim realized field advantage. The stronger interpretation is therefore that the heavier seeds of Kakunzu × Nyota provide a biologically plausible basis for enhanced early vigor that should be tested in future work rather than assumed as an already demonstrated outcome.

From a breeding perspective, the results underscore the value of integrating locally adapted landraces with improved cultivars. Kakunzu likely contributed adaptation to semi-arid production conditions, whereas Nyota represents the nutritional advantages

targeted in iron-biofortified bean breeding [4]. The present results suggest that using Kakunzu as the maternal parent is a more promising route for capturing favorable seed-size heterosis while maintaining a locally recognizable seed-coat phenotype. This may matter not only for crop performance but also for market acceptance, since seed appearance strongly influences bean market class and farmer preference in East Africa.

The manuscript also demonstrates the value of adding reproducible image analysis to reciprocal-cross studies. The original descriptive seed-coat observations became substantially stronger once lightness, maroon fraction, and maternal similarity were quantified. Although the seed-level image analysis was based on technical replicates extracted from the representative figure, it still improves transparency and reproducibility by making the maternal-effect argument measurable rather than purely visual.

## Conclusions

Reciprocal crossing between Kakunzu and Nyota revealed pronounced maternal effects on seed-coat phenotype and an asymmetric heterotic response for seed-size traits under semi-arid field conditions. Only the Kakunzu × Nyota hybrid expressed positive heterosis for seed width, area, and weight, with the strongest response observed for 4-seed weight. Image-based phenotyping confirmed that each reciprocal F1 resembled its maternal parent for seed-coat color composition. Together, these findings identify Kakunzu as the more favorable maternal parent for generating seed-trait advantages in this cross combination and provide a clear basis for further testing of early vigor, yield stability, and nutritional quality across seasons and locations.

## Study Limitations and Future Directions

This study was conducted in a single field environment, and the quantitative seed-coat image analysis used technical replicates derived from representative figure seeds rather than the full set of biological replicate images. Accordingly, the manuscript supports strong conclusions about reciprocal maternal effects on seed phenotype and seed-size heterosis, but more cautious conclusions about seedling vigor, drought resilience, and downstream yield advantages. Future work should evaluate emergence, seedling biomass, drought response, yield components, and iron concentration across multiple seasons and locations. Release-oriented breeding would also benefit from segregating-generation analysis and participatory evaluation with farmers in target dryland environments.

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