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Morphological Variation In Maize Ears From Reciprocal and Multi-Pollen Crosses

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Abstract. *Controlled pollination enables comparisons of ear traits among maize parental combinations, but differences in ear appearance do not necessarily establish genetic superiority or the origin of individual kernels. This study characterized the morphological responses of Pulut Uri (P), Srikandi Kuning (K), and Srikandi Ungu (U) using nine recorded pollination combinations in a randomized complete block arrangement with three replications at Universitas Teuku Umar, Indonesia, during November 2020–March 2021. Parental plant heights were documented at 15, 30, and 45 days after planting; ear weight with and without husks, kernel number, and kernel coloration were recorded for pollination treatments. The U♀ × P♂ combination produced the largest recorded mean ear weights with husks (236.67 g) and without husks (174.44 g), and its reported least significant difference groupings were highest for both traits. The same treatment had the largest numerical kernel count (300.89 kernels per ear), although no inferential separation for this trait was provided. Ear photographs showed cream, yellow, orange, red, and purple pigmentation patterns. Reciprocal directions differed descriptively in several pairs, whereas applications involving two pollen sources did not produce a consistently higher ear weight. These observations provide preliminary information for selecting parental combinations, while color phenotypes cannot establish the paternal origin of individual kernels.*

Keywords: ***Controlled pollination; Ear weight; Kernel pigmentation; Maize; Reciprocal crosses.***

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal for food, animal feed, and agricultural industry. Its improvement requires breeding strategies that consider both reproductive performance and grain characteristics. Although grain yield is ultimately determined by several interacting components, measurements made at the ear level are useful for initial characterization of breeding material. Ear traits are influenced by genetic background, plant growth, and the conditions surrounding flowering and kernel filling (Borrás & Vitantonio-Mazzini, 2018; Dong et al., 2023; Kosgei et al., 2025). Consequently, preliminary comparisons should distinguish observable ear phenotypes from validated estimates of yield potential or genetic merit.

Controlled pollination provides an operational means of studying specific female–male combinations. Its interpretation is especially important in maize because the ear develops on the maternal plant, while pollen contributes to the developing embryo and endosperm following fertilization. Reversing maternal and paternal roles can lead to different ear-level observations, but such differences may reflect the maternal plant, genetic interactions, or conditions of reproductive development rather than a single, isolated cause. Reciprocal crossing studies have shown that parental direction

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and maternal effects can contribute to variability in yield components (Antony John et al., 2024; Engida et al., 2024; Manigben et al., 2024). Thus, reciprocal comparisons can be informative even when the available data support only a descriptive interpretation.

Ear weight with husks and ear weight after husk removal describe overlapping but nonidentical attributes. The former includes covering tissues, while the latter more directly represents the harvested ear without those tissues; neither is equivalent to dry grain yield unless grain mass and moisture are separately determined. Kernel number reflects successful kernel establishment and is linked to silk development, pollination, fertilization, and resource availability. Subsequent filling depends on the balance between assimilate supply and reproductive demand (Borrás & Vitantonio-Mazzini, 2018; Fernández et al., 2022; Liu et al., 2022). These relationships provide a physiological rationale for reporting ear weight and kernel number together while avoiding the assumption that they respond identically.

Breeders also evaluate the visible characteristics of harvested grain. Yellow and orange maize pigments are often associated with carotenoid pathways, whereas red-to-purple coloration can involve anthocyanin biosynthesis and its regulation in different kernel tissues (Cai et al., 2023; Chen et al., 2024; Hu et al., 2023; Nie et al., 2024; Ran et al., 2026). Importantly, the genetic meaning of a visible color depends on whether pigmentation occurs in maternal tissues such as the pericarp or in tissues affected by fertilization. Pollen-source effects, including xenia, have been reported in maize; nevertheless, a photograph alone cannot determine the paternal genotype of an individual kernel (Suaib et al., 2020; Chatham & Juvik, 2021; Schoemaker et al., 2024).

A further conceptual distinction arises when more than one male variety supplies pollen to the same female treatment. This is not automatically equivalent to a conventional three-way hybrid, which is developed by a defined sequence of crossing generations, such as the use of an established single-cross hybrid as a parent in a subsequent cross (Kazem & Abed, 2026). When two sources of pollen are applied to one maternal ear, different kernels may have different pollen donors, and without further verification their parentage remains unresolved. For transparent reporting, the pollination arrangement should therefore be described by its recorded pollen sources rather than being presented as a genetically confirmed three-parent genotype.

Pulut Uri, Srikandi Kuning, and Srikandi Ungu provide contrasting maize materials for an exploratory assessment of ear morphology and kernel appearance. The available study records include six female–male combinations forming three reciprocal pairs and three additional entries involving two listed pollen sources. The central reporting need is to interpret these entries within the same framework without conflating parental growth, ear responses, and genetic inheritance. Accordingly, this study aimed (i) to describe plant height in the three parental varieties, (ii) to compare recorded ear-weight and kernel-number means among nine pollination treatments, (iii) to document visible kernel-color variation, and (iv) to discuss implications and limitations for preliminary parental selection.

MATERIAL AND METHODS

Study site, period, and parental materials

The study was conducted at the Experimental Farm, Faculty of Agriculture, Universitas Teuku Umar,

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Indonesia, between November 2020 and March 2021. Three named maize varieties were used as parental materials: Pulut Uri (P), Srikandi Kuning (K), and Srikandi Ungu (U). Each label in the pollination records identifies a seed-bearing female variety (♀) or a pollen-source male variety (♂). The identity of the seed-bearing plant is particularly important when interpreting ear-level traits, because an ear is physically produced and filled by the female plant.

Pollination treatments and experimental design

A non-factorial randomized complete block design was reported, comprising nine pollination treatments with three replications. Six treatments involved one female and one male source, including reciprocal directions for the pairs P–K, P–U, and K–U. The remaining three treatments listed one female variety and two pollen-source varieties. These three entries are referred to in this manuscript as two-source or multi-pollen applications; this terminology records the applied pollen-source category without assuming that kernels received genetic material from both male parents. Table 1 summarizes the recorded treatment identities.

Table 1. Female parents and recorded pollen sources in the nine maize pollination treatments

Treatment code	Female parent	Recorded pollen source(s)
P♀ × K♂	Pulut Uri	Srikandi Kuning
P♀ × U♂	Pulut Uri	Srikandi Ungu
K♀ × P♂	Srikandi Kuning	Pulut Uri
K♀ × U♂	Srikandi Kuning	Srikandi Ungu
U♀ × P♂	Srikandi Ungu	Pulut Uri
U♀ × K♂	Srikandi Ungu	Srikandi Kuning
P♀ × (K♂ + U♂)	Pulut Uri	Srikandi Kuning + Srikandi Ungu
K♀ × (P♂ + U♂)	Srikandi Kuning	Pulut Uri + Srikandi Ungu
U♀ × (P♂ + K♂)	Srikandi Ungu	Pulut Uri + Srikandi Kuning

Note. P = Pulut Uri; K = Srikandi Kuning; U = Srikandi Ungu. The plus sign designates two recorded pollen-source varieties in one treatment, not a demonstrated dual paternal contribution to the genome of an individual kernel.

Artificial pollination procedure

Artificial pollination was performed when maize silks were receptive and tassels were shedding pollen. In the study description, receptive female silks were recognized by their pale yellow appearance, while anthesis was identified from visible pollen release by the male inflorescence. Pollen from the designated male source or sources was manually transferred to the female silks according to the assigned treatment. As is typical of maize reproduction, close synchrony between pollen availability and silk receptivity is relevant to kernel establishment (Wang et al., 2022; Borrás & Vitantonio-Mazzini, 2018). The reported procedure does not distinguish whether the two pollen sources in multi-pollen treatments were applied simultaneously or in a specified sequence; hence, no sequencing mechanism is inferred from those labels.

Morphological observations

Two distinct groups of observations were reported. First, plant height (cm) was documented for each of the three parental varieties at 15, 30, and 45 days after planting (DAP). These measurements characterize parental vegetative growth and are not presented as plant heights of the nine pollination treatments. Second, the reproductive observations for each recorded pollination combination comprised ear weight with husks (g), ear

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weight after husk removal (g), and the mean number of kernels per ear. Visible kernel coloration was documented from photographs of the harvested ears. The photographs permit qualitative description of pigmentation but not quantitative pigment analysis, tissue-specific scoring, or genetic parentage determination.

Statistical reporting and scope of inference

The study reported analysis of variance using an F test, followed when appropriate by a least significant difference (LSD) test at $\alpha = 0.05$. Treatment means, LSD values, and letter groupings available for ear weights are reproduced without recalculation. The reported LSD was 21.56 g for ear weight with husks and 19.70 g for ear weight without husks. No corresponding mean-separation letters are available for kernel count or parental plant height in the reported tables. Accordingly, any rank differences for these variables are discussed only as numerical or descriptive patterns. The pollination treatments are compared for the studied location and growing period; observed means are not treated as evidence of stable hybrid performance across environments.

RESULTS AND DISCUSSION

Parental vegetative growth

All three parental varieties increased in height between 15 and 45 DAP (Table 2). At 15 DAP, Pulut Uri had a recorded mean of 29.01 cm, while Srikandi Kuning and Srikandi Ungu were 34.79 and 34.33 cm, respectively. The same numerical ordering was observed at 30 DAP, when heights were 82.29, 102.64, and 97.04 cm. By 45 DAP, Srikandi Kuning was tallest numerically (199.17 cm), followed by Srikandi Ungu (175.70 cm) and Pulut Uri (155.86 cm). These observations describe the growth of the parental plants and should not be used to infer either height heterosis or growth differences among the nine pollination combinations.

Table 2. Mean plant height of the three parental maize varieties at three observation dates

Parental variety	15 DAP (cm)	30 DAP (cm)	45 DAP (cm)
Pulut Uri (P)	29.01	82.29	155.86
Srikandi Kuning (K)	34.79	102.64	199.17
Srikandi Ungu (U)	34.33	97.04	175.70

Height is a relevant agronomic descriptor, but its relationship with ear performance is not sufficiently established by these tables. A taller maternal variety need not generate the heaviest ear under every pollen treatment, and the data do not provide plot-level pairing of vegetative and reproductive measurements to test such a relationship. Quantitative studies of maize traits show that plant architecture and yield components may be influenced by multiple loci and environmental interactions (Dong et al., 2023; Zhang & Qi, 2021; Zhang et al., 2025). Therefore, plant height in this study is best understood as background characterization of the parental varieties.

Ear weight with and without husks

Ear weight differed numerically across the nine treatments, with reported mean-separation groups available for both ear-weight measures (Table 3). U♀ × P♂ had the largest ear weight with husks (236.67 g; group f) and without husks (174.44 g; group f). In contrast, K♀ × P♂ recorded the smallest corresponding means (91.67 g; group a and 64.44 g; group a). The largest absolute values therefore occurred when Srikandi Ungu was the seed-bearing parent and Pulut Uri the pollen source. These results describe a favorable ear-weight phenotype for this particular combination in the reported experiment, not a measured increase in grain yield per unit land area.

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Table 3. Reported ear-weight and kernel-number means for nine maize pollination combinations

Pollination treatment	Ear weight with husks (g)	Ear weight without husks (g)	Kernels per ear
P♀ × K♂	120.22 b	90.89 b	175.11
P♀ × U♂	121.11 b	78.89 ab	106.78
K♀ × P♂	91.67 a	64.44 a	137.67
K♀ × U♂	200.00 e	152.22 e	252.22
U♀ × P♂	236.67 f	174.44 f	300.89
U♀ × K♂	177.00 d	133.33 d	276.22
P♀ × (K♂ + U♂)	175.56 d	126.11 cd	191.56
K♀ × (P♂ + U♂)	145.00 c	113.33 c	183.11
U♀ × (P♂ + K♂)	170.56 d	134.44 de	220.33

Note. Reported $LSD_{0.05}$ = 21.56 g for ear weight with husks and 19.70 g for ear weight without husks. Means sharing at least one letter within the same ear-weight column were not distinguished by the reported LSD procedure. No mean-separation letters were reported for kernel number; its ranking is descriptive.

Two complementary interpretations follow from the weight measurements. First, the rankings of the two ear-weight characters were broadly concordant: the treatment with the greatest mean including husks also produced the greatest mean after husk removal. Second, these are measurements of whole-ear components, not measurements of shelled kernel dry mass. Variation in cob size, husk contribution, kernel filling, and moisture may all influence measured ear weight, but the available measurements do not partition these components. Ear growth and kernel development depend on interacting source–sink processes and developmental timing (Borrás & Vitantonio-Mazzini, 2018; Liu et al., 2022; Wang et al., 2023). Consequently, whole-ear weight is useful for screening, although it cannot alone establish commercial productivity.

Descriptive contrasts between reciprocal directions

The six single-source treatments form three reciprocal pairs, enabling descriptive comparison of the two directions while keeping in mind that a different maternal variety develops each ear. For the P–K pair, P♀ × K♂ had a higher observed mean ear weight with husks than K♀ × P♂ (120.22 versus 91.67 g). For the P–U pair, U♀ × P♂ had a much higher recorded mean than P♀ × U♂ (236.67 versus 121.11 g). For the K–U pair, K♀ × U♂ exceeded U♀ × K♂ numerically for ear weight with husks (200.00 versus 177.00 g). Comparable directional patterns were visible in weight without husks. The magnitude and direction of these contrasts are based on recorded means and are not interpreted here as a separate statistical test of reciprocal effects.

These observations are biologically relevant because the maternal plant provides the ear tissues and supports seed development, whereas pollen participates in fertilization and may affect certain kernel tissues. Studies specifically addressing reciprocal crosses and maternal effects have reported variation in reproductive characters that depends on parental direction (Antony John et al., 2024; Bianchi et al., 2025). In the present experiment, however, the contrasting combinations do not isolate maternal physiology, pollen effects, or genotype-by-environment interactions. It is therefore more precise to describe direction-associated ear phenotypes than to attribute the contrasts to a verified genetic or cytoplasmic mechanism.

Responses in two-pollen-source treatments

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Three entries involved one maternal variety and two recorded sources of pollen. Their numerical outcomes did not follow a common pattern relative to the corresponding single-source treatments. On Pulut Uri ears, P♀ × (K♂ + U♂) had a mean weight with husks of 175.56 g, compared with 120.22 g for P♀ × K♂ and 121.11 g for P♀ × U♂. On Srikandi Kuning ears, K♀ × (P♂ + U♂) was intermediate at 145.00 g, compared with 91.67 g for K♀ × P♂ and 200.00 g for K♀ × U♂. On Srikandi Ungu ears, U♀ × (P♂ + K♂) recorded 170.56 g, below both U♀ × P♂ (236.67 g) and U♀ × K♂ (177.00 g). Thus, the recorded two-source treatment did not consistently increase whole-ear mass across maternal backgrounds.

This pattern emphasizes the importance of describing treatment operations accurately. The notation K♂ + U♂, for example, refers to two listed pollen sources applied to Pulut Uri female silks, not to a genetically validated three-way cross. In established three-way hybrid breeding, the crossing scheme proceeds through defined generations (Kazem & Abed, 2026). Because the paternal origin of individual kernels was not assessed in the present study, the results cannot demonstrate joint paternal contribution or relative fertilization success by the two male varieties. The treatment means remain valid morphological observations, but their genetic interpretation requires appropriate restraint.

Kernel number and its relationship to ear development

Kernel number varied numerically among the pollination entries (Table 3). U♀ × P♂ recorded 300.89 kernels per ear, followed by U♀ × K♂ (276.22) and K♀ × U♂ (252.22). The smallest mean was 106.78 kernels per ear for P♀ × U♂. Although these contrasts are substantial in numerical terms, the available result table does not provide mean-separation letters or an F statistic for kernel number. Accordingly, the highest value is a descriptive ranking and should not be described as a statistically demonstrated advantage.

Kernel set is determined during a developmentally sensitive period. Ear biomass accumulation, the emergence and receptivity of silks, synchronization with pollen shedding, and competition for assimilates can influence the number of kernels that ultimately develop (Borrás & Vitantonio-Mazzini, 2018; Fernández et al., 2022). Moreover, the same number of kernels need not produce the same whole-ear weight because grain filling and other ear tissues contribute to final mass. The observed co-occurrence of high kernel count and high ear weight in U♀ × P♂ is therefore consistent with potentially favorable reproductive development, but the experiment did not measure the physiological processes necessary to identify its cause.

Visible kernel-color diversity

The original photographs of the nine harvested ears show visible variation in pigmentation (Figure 1). Ears associated with Pulut Uri as the female source include pale yellow and cream kernels, with a mixed light-colored and reddish appearance in one single-source treatment. Ears involving Srikandi Kuning show predominantly yellow to orange appearances in the photographed set. Photographs with Srikandi Ungu as the female source include deep red-to-purple appearances. These are qualitative visual descriptors rather than a standardized color scale; the number or proportion of kernels of each color was not measured.

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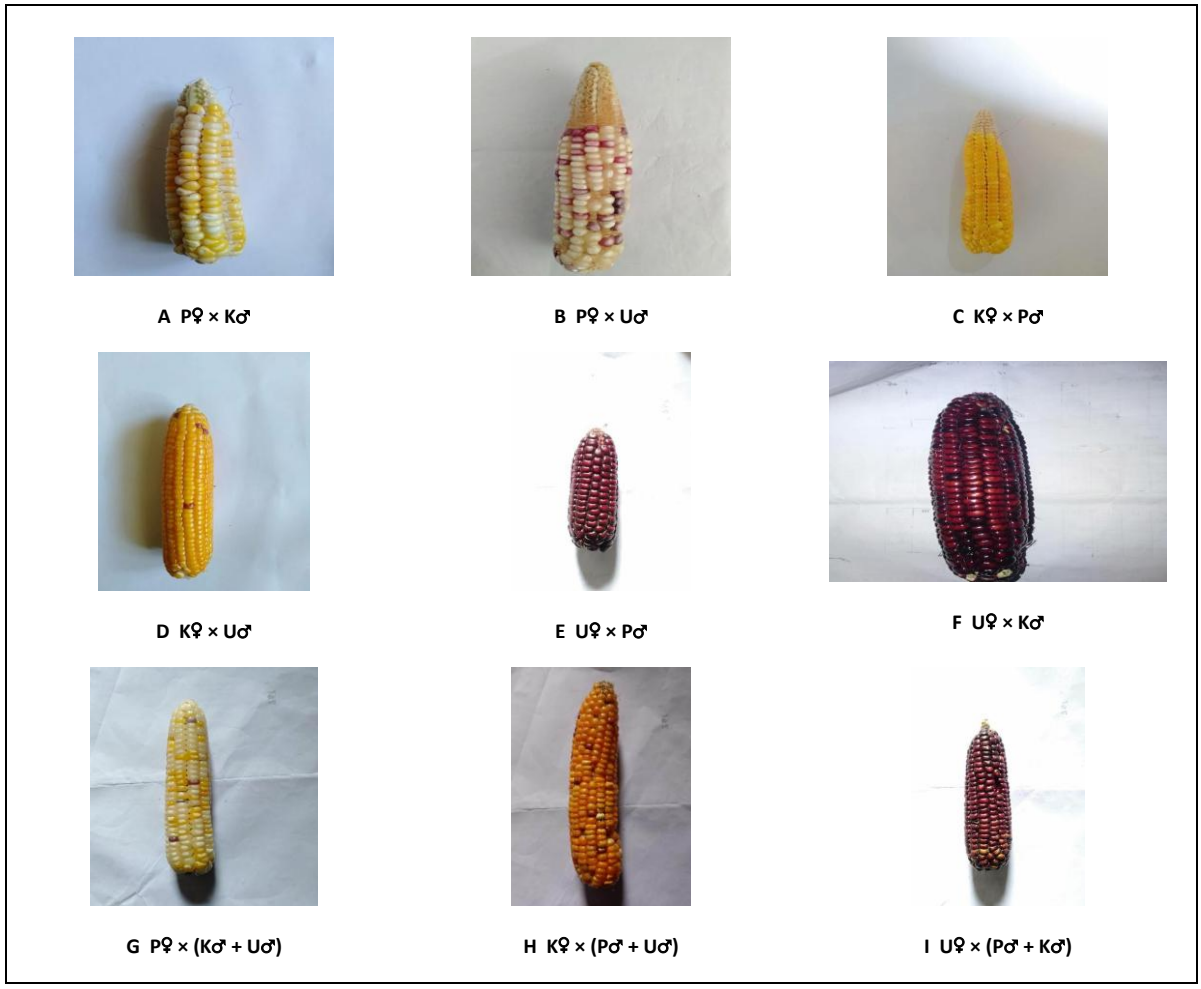


Figure 1. Original maize ears photographed under the nine recorded pollination treatments (A–I). Visual appearances are illustrative; image-based color cannot confirm pollen-donor identity or tissue-specific pigment composition.

Maize kernel color is controlled by pigment synthesis and its spatial distribution across tissues. Carotenoid biosynthesis contributes to yellow–orange pigmentation, while anthocyanins are associated with some red–purple phenotypes (Cai et al., 2023; Chen et al., 2024; Hu et al., 2023; Nie et al., 2024; Ran et al., 2026). Genetic studies have documented loci governing color traits and pigmentation intensity, yet different visible colors cannot be mapped to particular alleles from photographs alone (Schoemaker et al., 2024; Yin et al., 2024). The distinction between maternal pericarp and fertilization-derived endosperm is important because the appearance of these tissues can respond differently to pollen and maternal genotype (Suaib et al., 2020; Chatham & Juvik, 2021). The present photographs document phenotypic variation only and do not establish xenia in an experimentally isolated tissue or reveal the paternal identity of kernels in a two-source treatment.

Practical implications and study limitations

The strongest descriptive candidate from the current dataset is U♀ × P♂ because it combined the highest reported ear-weight means with the highest numerical kernel count. Its value lies in identifying a potentially informative female–male combination for further breeding consideration, rather than in

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demonstrating an already released superior hybrid. Breeding decisions would ordinarily account for grain yield, moisture-adjusted kernel mass, seed quality, repeatability across growing environments, and stability under stress. Multi-trait selection approaches similarly caution against ranking materials from one character or one trial alone (Ruswandi et al., 2023; Engida et al., 2024; Kosgei et al., 2025).

Three limitations govern interpretation. First, parental height measurements and reproductive pollination-treatment measurements describe different comparison sets and should not be treated as one common statistical factor. Second, the available reporting retains ear-weight LSD groupings but not inferential grouping information for kernel count; therefore, the latter remains a descriptive observation. Third, kernel color was photographed rather than quantified or genetically verified, and a two-pollen-source entry cannot be treated as a confirmed three-way hybrid. These boundaries do not negate the observed morphological differences. Instead, they define the contribution of the study as a field-based preliminary characterization of recorded maize pollination combinations, with a transparent basis for subsequent agronomic evaluation.

CONCLUSION

The recorded maize pollination combinations showed variation in ear weight, numerical kernel number, and visible pigmentation. The Srikandi Ungu female × Pulut Uri male combination had the highest reported means for both ear-weight traits and the greatest numerical kernel count within the observed set. Reciprocal directions differed descriptively, while applications involving two pollen sources did not consistently improve ear weight across maternal backgrounds. Parent-variety height and ear-response measurements represent distinct observation sets. These findings provide initial guidance for choosing combinations for further maize evaluation, but they do not by themselves establish superior field yield, the paternal genotype of individual kernels, or verified three-way hybrid formation.

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