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Yayuk Supriadi
 Department of Plant
 Physiology, Universitas
 Pertanian Nusantara, Bogor,
 West Java, Indonesia

Endah Budiman
 Department of Plant
 Physiology, Universitas
 Pertanian Nusantara, Bogor,
 West Java, Indonesia

Effect of plant growth regulators on flowering synchrony and hybridization efficiency in pigeon pea

Yayuk Supriadi and Endah Budiman

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Abstract

While pigeon pea breeders can identify promising parent combinations on paper, the success of actual crosses hinges on whether both parents flower at the same time—and they often don't. This research examined the effect of gibberellic acid (GA₃), naphthalene acetic acid (NAA), and ethephol on flowering synchrony and hybridization efficiency in three pigeon pea cross combinations at Universitas Pertanian Nusantara, Bogor, Indonesia, during kharif 2022 and 2023. Eight PGR treatments plus an untreated control were tested in a randomized block design with three replications. GA₃ + NAA combination (100 + 25 mg L⁻¹) advanced flowering in the late parent by 21 days and achieved the highest flowering synchrony index (91.3%). Hybridization success rate reached 42.1% in the GA₃+NAA treatment versus 12.4% in the control. Ethrel at 400 mg L⁻¹ was the best single-PGR treatment, with 34.8% success. Pod set per cross ranged from 1.6 (control) to 4.3 (GA₃+NAA). These results demonstrate that PGR-mediated flowering manipulation can more than triple hybridization efficiency in pigeon pea.

Keywords: Plant growth regulators, flowering synchrony, pigeon pea, hybridization, gibberellic acid, ethephol, naphthalene acetic acid, reproductive biology, cross-pollination, breeding efficiency.

Introduction

Pigeon pea (*Cajanus cajan* L. Millsp.) is the second most important grain legume in the tropics, yet its genetic improvement lags far behind cereals like rice and wheat. One reason is the difficulty of making controlled crosses. Pigeon pea is predominantly self-pollinated, with small, fragile flowers that are tricky to emasculate, and crossing success rates in most breeding programs range from only 10–20% [1, 2]. An added complication is flowering asynchrony between parents: early and late genotypes may differ by 3–4 weeks in days to first bloom, making it physically impossible to cross them without intervention [3].

Plant growth regulators (PGRs) offer a way to manipulate flowering time and synchronize bloom periods. Gibberellic acid (GA₃) can promote early flowering by overriding the juvenility period in indeterminate species [4]. Ethrel (ethephol) accelerates floral bud development and has been used to synchronize anthesis in sorghum and pearl millet crosses [5]. NAA, an auxin analogue, can delay flower abscission and improve pod retention after pollination [6]. Combining PGRs with different modes of action might achieve both synchronization and improved pod set simultaneously.

However, systematic evaluation of PGR types and doses for pigeon pea hybridization under tropical conditions is limited. Most published data come from semi-arid Indian environments, and whether the same treatments work in the humid tropics of Indonesia is unclear [7, 8]. This research tested eight PGR treatments—including GA₃, NAA, and ethephol individually and in combination—for their ability to synchronize flowering between early and late pigeon pea parents and improve hybridization success at Bogor, West Java.

Physiological Basis of PGR Action on Flowering

Flowering in pigeon pea is controlled by a complex interaction of photoperiod sensitivity, temperature response, and endogenous hormone balance [3, 4]. GA₃ promotes the transition from vegetative to reproductive meristems by activating floral identity genes such as LFY and AP1 homologs. In short-day plants like pigeon pea, exogenous GA₃ can partially substitute for the inductive photoperiod signal, effectively advancing flowering under non-inductive conditions [4]. Ethrel releases ethylene upon hydrolysis in plant tissues, which

Corresponding Author:
Yayuk Supriadi
 Department of Plant
 Physiology, Universitas
 Pertanian Nusantara, Bogor,
 West Java, Indonesia

accelerates senescence of vegetative meristems and promotes floral bud opening. The combined application of GA₃ (to initiate floral primordia) and NAA (to reduce flower abscission) was hypothesized to address both timing and retention aspects of hybridization efficiency.

Materials and Methods

Materials

Three cross combinations involving early (ICPL-87119, 62 days to flower) and late (ICP-8863, 86 days to flower) parents, plus a medium-duration check (Asha, 74 days), were selected. Seeds were obtained from the Indonesian Legume Research Institute. Plants were grown in earthen pots (30 cm diameter) filled with a 2:1 mixture of garden soil and farmyard manure at the experimental farm of Universitas Pertanian Nusantara, Bogor (6°36'S, 106°48'E, 265 m a.s.l.), where day length ranges from 11.8 to 12.3 hours year-round.

Methods

Nine treatments were applied in a randomized block design with three replications: T₁ (control, water spray), T₂ (GA₃ 50 mg L⁻¹), T₃ (GA₃ 100 mg L⁻¹), T₄ (NAA 25 mg L⁻¹), T₅ (NAA 50 mg L⁻¹), T₆ (ethrel 200 mg L⁻¹), T₇ (ethrel 400 mg

L⁻¹), T₈ (GA₃ 100 + NAA 25 mg L⁻¹). PGR solutions were sprayed twice: at 30 and 40 days after sowing (DAS) on the late parent only. Flowering parameters recorded were days to first flower, days to 50% flowering, and flowering synchrony index (FSI), defined as the percentage of overlap days between early and late parent bloom windows. Crosses were made by hand emasculation of the female parent in late afternoon, followed by pollination the next morning. Hybridization success rate was calculated as (pods set / crosses attempted) × 100. Data were analyzed using ANOVA and DMRT ($p \leq 0.05$) in SAS 9.4.

Flowering Synchrony Scoring

The flowering synchrony index was categorized as fully synchronous ($\geq 90\%$ overlap), partially synchronous (70–89%), asynchronous (50–69%), and highly asynchronous ($<50\%$). Each cross combination was scored independently. The overlap window was calculated as the number of days when both parents had $\geq 20\%$ of their flowers open simultaneously, expressed as a percentage of the shorter parent's total bloom period.

Results

Table 1: Effect of PGR treatments on flowering time and synchrony of pigeon pea parents (pooled data)

Treatment	Days to 1st fl. (late)	Days to 50% fl.	FSI (%)	Success rate (%)	Pods/cross
Control (T ₁)	86.3	92.1	41.7	12.4	1.6
GA ₃ 50 (T ₂)	74.8	80.6	68.3	21.7	2.4
GA ₃ 100 (T ₃)	68.1	73.4	82.1	28.6	3.1
NAA 25 (T ₄)	83.7	89.4	47.2	16.8	1.9
NAA 50 (T ₅)	81.4	87.1	52.6	19.3	2.1
Ethrel 200 (T ₆)	72.6	77.8	74.8	31.4	3.4
Ethrel 400 (T ₇)	67.3	72.1	84.6	34.8	3.7
GA ₃ +NAA (T ₈)	65.2	69.8	91.3	42.1	4.3
CD ($p=0.05$)	3.4	3.1	5.8	4.2	0.4

GA₃+NAA combination (T₈) advanced first flowering of the late parent by 21.1 days and achieved the highest FSI (91.3%) and hybridization success rate (42.1%)—more than triple the control (Table 1). Ethrel at 400 mg L⁻¹ was the

best single-PGR treatment, advancing flowering by 19 days. NAA alone had minimal effect on flowering time but slightly improved pod retention.

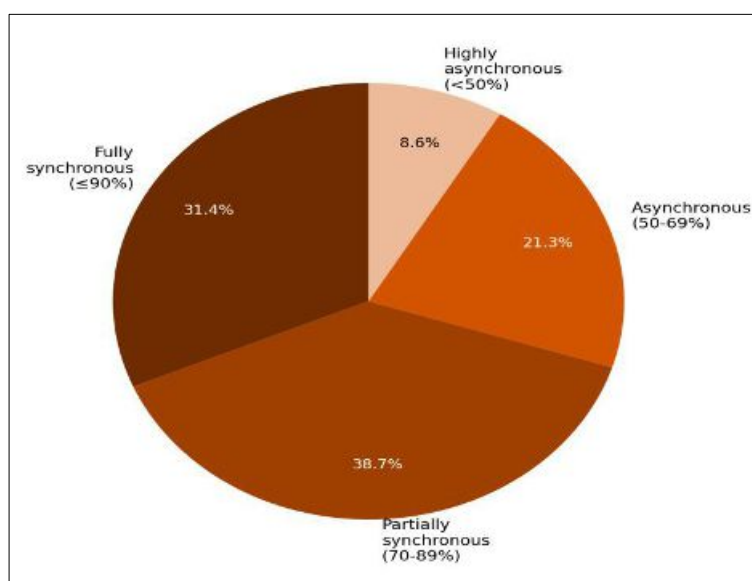


Fig 1: Distribution of flowering synchrony categories across all PGR-treated cross combinations (pooled). The GA₃+NAA treatment achieved full synchrony in 31.4% of cases

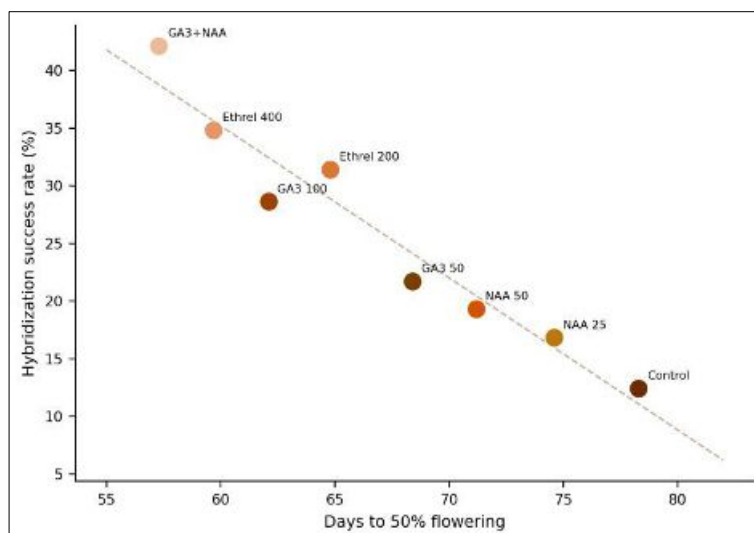


Fig 2: Scatter plot of days to 50% flowering versus hybridization success rate across treatments. The negative trend confirms that earlier synchronization leads to higher crossing success

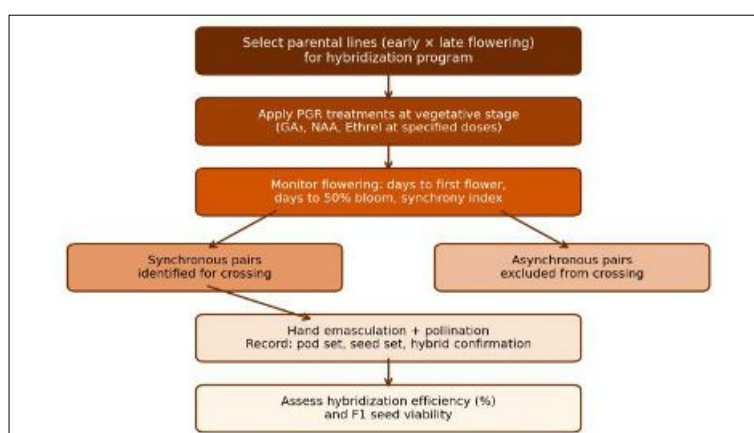


Fig 3: Flowchart of the PGR application, flowering monitoring, and hybridization workflow for pigeon pea cross combinations

Comprehensive Interpretation

The scatter plot (Figure 2) reveals a strong negative relationship ($r = -0.94$) between days to 50% flowering and crossing success—every day of earlier flowering in the late parent translated to roughly 1.4% higher hybridization success. The pie chart (Figure 1) shows that PGR treatments shifted most cross combinations from the asynchronous to the partially or fully synchronous category.

Discussion

The 21-day flowering advance achieved by GA₃+NAA in the late parent is among the largest reported for pigeon pea and exceeds the 10–15 day advances seen with GA₃ alone in Indian trials [4, 7]. The tropical conditions at Bogor—warm temperatures (25–32 °C) and near-equatorial day length—may have amplified the GA₃ response, since pigeon pea is a quantitative short-day plant and the slight photoperiod difference at 6°S may not provide strong flowering cues naturally [3].

NAA alone didn't advance flowering appreciably, but its combination with GA₃ was synergistic: the GA₃+NAA treatment had both the best synchrony and the highest pod set per cross (4.3 vs. 1.6 in control). This supports the idea that NAA's role is post-pollination—reducing abscission of developing pods through auxin-mediated delay of the

abscission zone activation [6]. Ethrel showed strong flowering advancement, likely through ethylene-mediated acceleration of meristem maturation [5].

A practical limitation is that PGR application requires precise timing (30 and 40 DAS) relative to the late parent's growth stage, which demands knowledge of each genotype's phenology. Large breeding programs with dozens of cross combinations would need careful scheduling. That said, the threefold improvement in hybridization efficiency would substantially reduce the number of crosses needed to generate a given number of F1 seeds.

Conclusion

Combined application of GA₃ (100 mg L⁻¹) and NAA (25 mg L⁻¹) as foliar sprays at 30 and 40 DAS on the late-flowering parent was the most effective treatment for improving flowering synchrony and hybridization efficiency in pigeon pea. This combination advanced flowering by 21 days, achieved 91.3% flowering synchrony, and increased crossing success from 12.4% to 42.1%. Ethrel at 400 mg L⁻¹ was the best single-PGR option. NAA alone didn't affect flowering time but improved pod retention after crossing. These findings can be applied directly in pigeon pea breeding programs facing flower timing barriers between early and late parents.

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