



Research article

Comparison of self-pollination and intercrossing for feminized seed production in Thai Hang Kra Rok cannabis (*Cannabis sativa* L.) landrace

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Article Info

Article history:

Received 7 March 2026

Revised 28 June 2026

Accepted 10 July 2026

Available online 30 September 2026

Keywords:

Colloidal silver,
Plant factory with artificial lighting
(PFAL),
S₁ generation,
Sex reversal,
Thai cannabis landrace

Abstract

Importance of the work: Often, growing 'Hang Kra Rok' cannabis conventional seeds results in unwanted male plants, which substantially reduce cultivation efficiency.

Objectives: To compare self-pollination and intercrossing strategies for producing high-quality feminized seeds using two distinct Thai landrace accessions.

Materials and Methods: Female cuttings from Bangkok and Sakon Nakhon accessions were treated with 30 parts per million colloidal silver to induce male flowers. Selfed (S₁) and hybrid (F₁) progeny were produced via manual pollination. S₁ and F₁ progenies were produced and evaluated in a plant factory with artificial lighting (PFAL) system.

Results: The S₁ progeny (Self-BKK and Self-SKN) and the F₁ hybrid (SKN×BKK) produced 100% female plants. In contrast, the F₁ hybrid (BKK×SKN) produced a single hermaphroditic plant, accounting for 5.6% of the population. Regarding vegetative growth, the F₁ progeny had significantly ($p < 0.05$) greater plant height than the S₁ progeny. Furthermore, flowering onset in the F₁ progeny was delayed by approximately 7 d. However, no significant differences were observed in dry inflorescence weights across all treatments. In terms of phytochemical quality, the S₁ progeny from the Bangkok source (Self-BKK) had the highest average Δ^9 -tetrahydrocannabinol (THC) content (6.58%), while the cannabidiol content did not differ significantly among treatments.

Main finding: Under controlled PFAL conditions, self-pollination was an effective strategy for maintaining sexual stability and achieving early flowering in these Thai cannabis landraces, without compromising yield or THC quality.

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<https://doi.org/10.34044/j.anres.2026.60.5.12>

Introduction

Cannabis sativa L. is a globally cultivated multipurpose crop valued for its bioactive cannabinoids, particularly Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), which underpin its pharmacological and industrial significance (Andre et al., 2016). Extensive phytochemical and genetic variation within the species have led to the classification of cannabis germplasm into distinct chemotypes based on cannabinoid composition and into biotypes characterized by morphological differentiation (Hillig, 2004; 2005). Furthermore, genome-wide analyses have demonstrated pronounced genetic structuring between drug-type and fiber-type populations, reflecting divergent domestication and breeding histories (Sawler et al., 2015). Typically, Southeast Asian drug-type landraces, including traditional Thai germplasm, are characterized as narrow-leaflet forms associated with THC-dominant chemotypes (Hillig, 2004; Sawler et al., 2015).

In commercial drug-type production, unpollinated female inflorescences are preferred because cannabinoid-rich glandular trichomes develop predominantly on female flowers (Andre et al., 2016; Punja and Holmes, 2020). *Cannabis sativa* L. Uses an XX/XY system for chromosomal sex determination, with females carrying XX chromosomes and males carrying XY chromosomes (Divashuk et al., 2014). Consequently, conventional seed production results in the segregation of male and female progeny in accordance with Mendelian inheritance (Divashuk et al., 2014). The presence of male plants is undesirable in drug-type cultivation as pollination initiates seed formation, alters floral morphology and reduces both inflorescence quality and cannabinoid yield (Punja and Holmes, 2020).

To circumvent male segregation, chemical induction of male flowers on genetically female plants has been adopted widely in feminized seed production systems (Ram and Sett, 1982; Lubell and Brand, 2018). Silver-based compounds, such as silver nitrate, silver thiosulfate (STS) and colloidal silver, are used commonly to inhibit ethylene action, thereby inducing fertile male flowers on XX female plants (Ram and Sett, 1982; Divashuk et al., 2014). Subsequently, Lubell and Brand (2018) reported consistent induction of male flowers and reliable feminized seed production following silver thiosulfate treatment in hemp. In addition to silver-based compounds, plant growth regulators can influence sex expression, with gibberellins promoting male flower formation, whereas abscisic acid partially suppresses this effect (Ram and Jaiswal, 1972).

Additionally, feminized seed production may arise through spontaneous hermaphroditism in genetically female plants (Ram and Sett, 1982; Punja and Holmes, 2020). Under specific environmental conditions or during late flowering stages, female plants may develop male flowers and the resulting pollen is similarly derived from an XX genetic background (Ram and Sett, 1982; Divashuk et al., 2014). Although generally, such pollination produces predominantly female progeny (Lubell and Brand, 2018), hermaphroditic expression has been associated with genetic variability and instability in progeny sex ratios, which may limit its suitability as a controlled seed production strategy (Punja and Holmes, 2020).

In commercial practice, silver-based compounds such as (STS) and colloidal silver are used commonly to induce male flower formation (Ram and Sett, 1982; Flajšman et al., 2021). Silver ions inhibit ethylene action in plant tissues (Beyer, 1976). Ethylene perception operates through receptor-mediated signaling pathways that regulate diverse developmental processes (Chen, 2005) and disruption of ethylene signaling has been associated with male floral induction in *C. sativa* (Beyer, 1976; Ram and Sett, 1982). Induced male flowers produce viable pollen suitable for controlled feminized seed production (Ram and Sett, 1982; Flajšman et al., 2021).

C. sativa is a dioecious and predominantly outcrossing species, for which genomic and transcriptomic resources have been established (Van Bakel et al., 2011) and population genomic studies have revealed substantial genetic diversity and structured variation among cultivated accessions (Sawler et al., 2015), while in addition, genomic variation, including gene copy number variation, has been associated with differences in phytochemical profiles (Vergara et al., 2019). Nevertheless, intensive selection and restricted effective population sizes in breeding programs may alter allele frequencies and reduce genetic variability through genetic drift and bottleneck effects (Nei et al., 1975). Furthermore, evolutionary genetic theory indicates that populations with histories of bottlenecks or partial inbreeding may differ in the magnitude of inbreeding depression depending on demographic history and the potential purging of deleterious alleles (Lande and Schemske, 1985; Charlesworth and Willis, 2009). Despite the increasing commercial importance of Thai drug-type landraces, empirical information remains limited on the genetic consequences of self-pollination versus cross-pollination in feminized seed production.

Thai 'Hang Kra Rok' landraces represent a critical genetic resource that lacks empirical data regarding the genetic consequences of self-pollination (S_1) versus intercrossing (F_1) in these landraces, particularly concerning inbreeding depression or sexual instability. Therefore, the present study evaluated the performance and stability of selfed and cross-pollinated progeny within a plant factory with artificial lighting (PFAL) system to determine the most effective breeding strategy for feminized seed production.

Materials and Methods

Plant material and origin

Female 'Hang Kra Rok' plants were sourced from two geographically and genetically distinct accessions—Bangkok (BKK), Central Thailand and Sakon Nakhon (SKN), Northeast Thailand—representing different regional adaptations of the same landrace (Fig. 1). Healthy, vigorous mother plants with no signs of pests or disease were selected as parental materials.



Fig. 1 Morphological characteristics of 'Hang Kra Rok' cannabis landraces from two genetic sources: (A) Bangkok and (B) Sakon Nakhon, where measurement scale is in centimeters

Experimental conditions

The experiment was conducted in a PFAL system, with the cultivation process divided into two phases: vegetative and reproductive. During the vegetative phase (long-day condition), plants were grown under a photoperiod of 18 hr of light daily, with a photosynthetic photon flux density (PPFD) maintained at 400–600 $\mu\text{mol}/\text{m}^2/\text{s}$. For the reproductive phase (short-day condition), the photoperiod was adjusted to 12 hr of light daily, with an increased PPFD of 800–1,200 $\mu\text{mol}/\text{m}^2/\text{s}$. The ambient air temperature was maintained at 24–26°C during the photoperiod and at 18–20°C during the dark period. Initially, the relative humidity (RH) was maintained below 60% and then reduced to below 55% during the reproductive phase using a dehumidification system. Throughout the reproductive stage, uniform air circulation within the plant canopy was ensured using oscillating fans. During the vegetative and reproductive stages, plants were supplied with a nutrient solution formulated according to the guidelines of Sreewongchai et al. (2022). To ensure high environmental uniformity, all environmental factors, including light intensity, temperature and nutrient delivery, were maintained consistently across the entire cultivation area using high-precision control systems, ensuring that all the studied plants received uniform growth conditions.

Induction of hermaphroditic flowers and seed production

Plant propagation

'Hang Kra Rok' cannabis plants were propagated via stem cuttings. In each accession, healthy shoots (10–15 cm in length) were harvested from the same mother plants. Leaves were trimmed to reduce transpiration and the bases were cut at a 45° angle to maximize the rooting area. A rooting hormone gel, indole-3-butyric acid (0.31% weight per volume), was applied to the base before the cuttings were inserted into Sublime growing medium (BVB Substrates; Netherland). The cuttings were maintained under long-day conditions in a propagation chamber with consistent moisture. Ventilation until root

initiation was provided once daily for 15 min by opening the chamber lid. After 2 wk, rooted cuttings were transplanted into pots (15 cm in diameter) containing coco peat. The planting holes were pretreated with 5 g per hole of Trichoderma powder (TRI-TAB; TAB Innovation Co., Ltd., Thailand) to prevent root rot. Then, the plants were moved to the vegetative growth stage under long-day conditions.

Sex reversal induction

At age 4 wk, three plants per accession were selected for sex reversal induction (pollen donors), while six plants per accession were designated as pollen recipients (female plants). Pollen donor female inflorescences were observed at 2 wk after the onset of short-day conditions. Then 1 wk later, sex reversal was induced by applying a foliar spray of 30 parts per million colloidal silver at a rate of 5 mL per inflorescence. The spray was applied daily to the tops of primary inflorescences during 1000–1100 hours for 3 wk. Then, at 1 wk after the final application, male flowers developed on the female inflorescences, providing viable pollen for the breeding process.

Pollination and seed development

To ensure synchronized flowering, the short-day treatment for pollen recipients was delayed by 5 wk relative to that of the pollen donors. By week 8, male flowers began anthesis. Controlled pollination was performed daily during 1000–1100 hours for 2 wk. Self-pollination was facilitated by gently shaking the plants to disperse pollen onto the female flowers in the same plant. For cross-pollination, pollen from three induced male donor plants per treatment was collected and applied to the target female plants with a brush. To prevent unintended cross-contamination, experimental units were isolated using fabric barriers. Following pollination, seeds were allowed to mature for 6 wk before being harvested for subsequent evaluation of the progenies.

Evaluation of S_1 and F_1 progeny

Experimental design

The S_1 progenies were generated through self-pollination using induced pollen on the selected female clones, while F_1 progenies resulted from reciprocal crosses. The experiment was evaluated using a completely randomized design (CRD) with four treatments, three replications and six plants per replication. The treatments were defined as follows: Self-BKK (S_1), Self-SKN (S_1), BKK×SKN (F_1) and SKN×BKK (F_1).

Germination and Seedling Establishment

For each treatment, 200 seeds were placed between moist paper towels and hydrated using reverse osmosis water. The seeds were incubated in germination boxes under controlled temperature and humidity. After 48 hr, seeds exhibiting radicle emergence were selected for transplanting. One seed was sown per 15 cm pot (60 pots per treatment) using coco peat with Trichoderma powder as the growing medium. From these, 18 uniform, healthy plants per treatment (six plants per replication) were selected for data collection on sex expression and growth parameters.

Vegetative management

Plants were maintained under long-day conditions for the vegetative phase. The nutrient solution was maintained at an electrical conductivity (EC) of 1.0 mS/cm and a pH of 6.2. By week 4, morphological measurements, including plant height, were recorded. Prior to the induction of flowering, 48 uniform, healthy plants per treatment were selected for the reproductive phase.

Reproductive phase and sex determination

The reproductive phase was induced by shifting to a short-day photoperiod (12 hr light). During this phase, the nutrient EC was increased to 1.2 mS/cm. In the first week, nylon netting (10 cm × 10 cm mesh) was installed to support the canopy. By the second week, sex expression and days to flowering were recorded. The nutrient solution was adjusted in week 6 to an EC of 1.4 mS/cm.

Harvesting and maturity indicators

Trichome development was monitored weekly from week 11 to harvest using a Wi-Fi portable digital microscope (1,000×; Shenzhen Joyhonest Technology Co., Ltd., Shenzhen, China). The optimal harvest window was determined when the trichome ratio reached 70–75% milky to 25–30% amber. Then at 1 wk prior to harvest, a nutrient flushing technique was applied using a potassium-rich, low-EC (0.2 mS/cm) solution at pH 6.2 to facilitate final maturation. The inflorescences were harvested using the wet trimming method, where inflorescences were immediately manicured, separated into smaller inflorescences and stripped of fan leaves.

Data collection

Plant height

The height of individual plants in each treatment was measured during the vegetative phase, prior to the transition to the reproductive phase. Measurements in centimeters were taken using a standard ruler from the surface of the growing medium to the apical meristem.

Sex expression

Sex expression was monitored in individual plants in each treatment during the reproductive phase. Data were recorded daily after the transition to a short-day photoperiod. Sex identification was conducted using a magnifying lens to examine reproductive organs at the apical and axillary buds. Plants were categorized based on the presence of pistils (female), pollen sacs (male), or both (hermaphrodite). Results were recorded as the number of plants per sex for each treatment.

Days to flowering

The number of days to flowering was recorded for each individual plant in each treatment. Observations were performed using a magnifying lens at the apical and axillary buds every day of the short-day period. Flowering onset was defined as the first visible appearance of pistils at the apical or axillary buds, verified daily. It was recorded as the total number of days from the first day of short-day induction to the initial appearance of floral structures.

Fresh and dry inflorescence weight

Plants were harvested individually at different times based on specific maturity thresholds—determined when the glandular trichome ratio reached 70–75% milky to 25–30% amber. This individualized harvest approach ensured that chemical accumulation was evaluated at the peak physiological maturity for each treatment. Post-harvest processing followed the wet trimming method. The fresh weight was measured using a digital balance and recorded in grams per plant. Then, the trimmed inflorescences were dried in a dark room at 20–24°C with the RH below 60%. After 10 d of drying, which reduced the final moisture content to approximately 9.5%, the dry inflorescence weight was measured for each plant. Then, these data were used to calculate the mean weight for each treatment.

Δ9-Tetrahydrocannabinol and cannabidiol contents analysis

In total, 36 dried inflorescence samples (three samples per replication) were selected randomly for cannabinoid analysis. For each sample, 2 g of dried inflorescence was ground finely to ensure homogeneity and stored in a plastic zip-lock bag to prevent contamination and moisture absorption. Analysis of each sample was conducted in three technical replicates using near-infrared spectroscopy (NIR) with a PerkinElmer Spectrum Two N, provided by LABACTIVE CO., LTD, (Thailand). The manufacturer's proprietary calibration model was validated using high-performance liquid chromatography for drug-type *C. sativa* samples. The calibration model had high predictive accuracy, with the coefficient of determination > 0.95 and the standard error of prediction within ± 1.040% and ± 1.106% for the total THC and total CBD, respectively. The analysis utilized the manufacturer's proprietary calibration model for cannabis constituents. Ground samples were packed tightly into a glass sample plate, ensuring uniform distribution and eliminating air gaps to optimize infrared light penetration. The results were reported as total cannabinoid percentages, consisting of total THC (the sum of THC and tetrahydrocannabinolic acid) and total CBD (the sum of CBD and cannabidiolic acid).

Statistical analysis

The mean value of each replicate (average of 6 plants) was used as the experimental unit for statistical analysis. Data were analyzed using one-way analysis of variance, consistent with the completely randomized design (CRD), to test for treatment effects at $p < 0.05$. Mean comparisons among treatments were performed using the least significant difference (LSD) test ($p < 0.05$). All statistical analyses were conducted using the R software (version 4.5.1; R Core Team, 2025) and RStudio (version 2026.04.0; Posit Team, 2026).

For sex expression data, the proportions of plants exhibiting each sex expression were calculated descriptively, and Fisher's exact test was used to test for differences in hermaphroditic expression among treatments.

Ethics statements

All research procedures involving were obtained and handled in compliance with Thai narcotics regulations (Ministry of Public Health Notification No. 9/2564) under institutional approval from the Cannabis, Hemp and Kratom Policy Committee, Kasetsart University, Bangkok, Thailand (Approval No. MHESI 6501.2102/ 4728, dated May 13, 2020).

Results

Plant height

The average plant height measured during the vegetative phase, prior to the reproductive phase, differed significantly among the four treatments. The F_1 progenies, SKN×BKK and BKK×SKN, had significantly greater mean heights of 87.22 and 88.78 cm, respectively. In contrast, the S_1 progenies had significantly lower mean heights, with Self-BKK and Self-SKN having 78.94 and 74.83 cm, respectively (Table 1). Based on these results, there was a clear trend of increased plant height in the crossing progenies compared to the self-pollination progenies in this germplasm.

Table 1 Mean plant height, days to flowering, and fresh and dry inflorescence weight of S_1 and F_1 progenies ($n = 3$ replicates per treatment; 6 plants per replicate) derived from self-pollination and inter-accession crossing, respectively, of 'Hang Kra Rok' cannabis landraces.

Treatment	Plant height (cm)	Time to flowering (d)	Fresh inflorescence weight (g/plant)	Dry inflorescence weight (g/plant)	THC content (total THC %)	CBD content (total CBD %)
S_1 : Self-BKK	78.94±5.17 ^b	28.17±0.7 ^b	64.75±32.25	13.35±6.63	6.58±1.36 ^a	0.00
S_1 : Self-SKN	74.83±8.26 ^b	28.89±1.08 ^b	53.17±22.70	10.82±4.9	5.06±0.56 ^b	0.22±0.05
F_1 : BKK×SKN	88.78±5.55 ^a	34.72±1.41 ^a	60.34±20.8	12.09±4.16	5.22±0.38 ^b	0.09±0.04
F_1 : SKN×BKK	87.22±4.57 ^a	35.83±0.86 ^a	57.55±19.85	11.41±4.12	5.05±0.71 ^b	0.16±0.03
F test	*	*	ns	ns	*	ns

THC = Δ^9 -tetrahydrocannabinol; CBD = cannabidiol. Values (mean $\pm$ SD) with different lowercase superscripts within the same column are significantly different, according to least significant difference test ($p < 0.05$). * = significant treatment effect at $p < 0.05$; ns = nonsignificant treatment effect ($p \geq 0.05$).

Table 2 Sex expression in S_1 and F_1 progenies ($n=18$ per treatment) derived from self-pollination and inter-accession crossing, respectively, of 'Hang Kra Rok' cannabis landraces.

Treatment	Female plants (N)	Hermaphrodite plants (N)	Female (%)
S_1 : Self-BKK	18	0	100.00
S_1 : Self-SKN	18	0	100.00
F_1 : BKK×SKN	17	1	94.44
F_1 : SKN×BKK	18	0	100.00

Sex expression

The phenotypic sex expression of 'Hang Kra Rok' cannabis S_1 and F_1 progenies varied across the experimental treatments (Table 2). Due to the 100% sexual stability observed in several treatments, the zero-variance precluded formal categorical testing; consequently, a descriptive proportion analysis was utilized to assess sex expression. However, Fisher's exact test was applied to evaluate the distribution, indicating no significant difference in the proportion of hermaphroditism between the treatments. The S_1 progenies derived from self-pollination, Self-BKK and Self-SKN, as well as the F_1 progenies of the SKN×BKK cross-pollinated hybrid, achieved a 100% female population, with no hermaphrodite plants observed. In contrast, the F_1 progenies of the BKK×SKN cross-pollinated group produced a single hermaphrodite plant, representing a 5.56% hermaphroditism rate. Consequently, the female plant ratio for this treatment was 94.44%.

Days to flowering and harvest maturity

Based on the results, the days to flowering of the 'Hang Kra Rok' cannabis S_1 and F_1 progenies differed significantly among treatments (Table 1). The F_1 progenies of SKN×BKK and BKK×SKN had a substantially longer vegetative duration before flowering, with mean onset times of 35.83 d and 34.72 d, respectively. In contrast, the S_1

progenies Self-BKK (28.17 d) and Self-SKN (28.89 d) were not significantly different but reached the flowering stage significantly earlier than the cross-pollinated groups. In addition, these S₁ progenies reached harvest maturity more quickly (119–120 d) than the F₁ progeny (126–128 d).

Fresh and dry inflorescence weights

There were no significant differences among the treatments regarding both fresh and dry inflorescence weights of the 'Hang Kra Rok' cannabis S₁ and F₁ progenies (Table 1). The average fresh inflorescence weight per plant was in the range 53.17–64.75 g, while the average dry inflorescence weight was in the range 10.82–13.35 g. Based on these results, the breeding strategy, whether self-pollination or cross-pollination, did not substantially influence the final inflorescence yield under the controlled PFAL conditions in the present study.

Δ9-Tetrahydrocannabinol and cannabidiol contents

The analysis of the cannabinoid profiles in the dried inflorescence of 'Hang Kra Rok' S₁ and F₁ progenies revealed that the total THC content differed significantly among treatments (Table 1). The self-pollinated Bangkok accession (Self-BKK) had the highest mean total THC concentration (6.58%), which was significantly greater than for all the other breeding groups. In contrast, the self-pollinated Sakon Nakhon strain (Self-SKN) and the cross-pollinated hybrids (BKK × SKN and SKN × BKK) had mean total THC levels in the range 5.05–5.22%, with no significant differences among them. The total CBD content concentrations remained low across all treatments (0.00–0.22%), where the reported value of 0.00% indicated that any concentration was below the detection limit in the NIR analysis. No significant variation was observed among the experimental groups

Discussion

Breeding strategy clearly influenced sexual stability and vegetative performance in the 'Hang Kra Rok' landrace. Self-pollinated progenies produced exclusively female plants, whereas a low frequency of hermaphroditism was detected in one cross-pollinated combination. In *C. sativa*, sex expression is governed by an XX/XY chromosomal system (Divashuk et al., 2014), yet phenotypic sex expression is not strictly fixed and may be modified by genetic background and environmental conditions (Punja and Holmes, 2020). Furthermore experimental evidence has demonstrated that sex expression in cannabis can be influenced by hormonal regulation and genotype-dependent responsiveness (Ram and Jaiswal, 1972). Therefore, the appearance of hermaphroditic individuals in the cross-derived progeny likely reflected altered genetic interactions resulting from recombination between differentiated germplasm pools rather than intrinsic instability in the landrace. Genome-wide analyses have revealed substantial genetic differentiation among cannabis accessions, such as Sawler et al. (2015), supporting the hypothesis that recombination between divergent lineages may disrupt co-adapted gene complexes involved in stable sex expression. The high female stability

observed in the S₁ progenies indicated that the 'Hang Kra Rok' landraces in the present study may tolerate self-pollination.

The cross-pollinated progenies had greater plant height than the self-pollinated progenies, indicating enhanced vegetative growth following hybridization. Increased vegetative performance in hybrids has been attributed commonly to heterosis, which may arise through dominance, overdominance, or epistatic interactions among divergent alleles (Birchler et al., 2010; Chen, 2013). At the molecular level, heterosis has been associated with altered gene expression patterns and regulatory network reprogramming resulting from allelic interactions between parental genomes (Chen, 2013). Given the documented genetic differentiation among cannabis germplasm groups (Sawler et al., 2015), combining distinct genetic backgrounds in the present study likely enhanced vegetative vigor by complementing partially deleterious alleles or through non-additive gene action.

However, greater vegetative vigor did not translate into earlier flowering. The cross-derived progenies flowered significantly later than self-pollinated lines, indicating that enhanced vegetative growth was not directly associated with accelerated reproductive transition under the present tested conditions. Flowering in *C. sativa* is known to be responsive to photoperiod and environmental cues (Spitzer-Rimon et al., 2019). Because all treatments in the present study were cultivated under identical PFAL conditions, including controlled photoperiod and environmental parameters, the delayed flowering observed in the hybrids likely reflected intrinsic genetic differences rather than environmental variation. Increased vegetative vigor may have prolonged the vegetative phase prior to floral transition—a pattern that may be associated with altered developmental regulation under heterosis (Chen, 2013). In PFAL systems, light management has a strong influence on biomass accumulation and yield potential (Rodriguez-Morrison et al., 2021). Therefore, variation in flowering time among genotypes may have practical implications for production planning, although this aspect warrants further investigation. While the F₁ hybrids had higher vegetative vigor, the associated delay in flowering and harvest suggested a potential trade-off in commercial efficiency. In standardized PFAL systems, longer vegetative and reproductive periods may translate into increased resource utilization, suggesting implications for operational efficiency in closed-loop systems and warranting further direct economic assessment. This reinforces the idea that self-pollination (S₁) may be more economically viable for high-turnover indoor production.

Notably, no evidence of inbreeding depression was detected in the self-pollinated groups for plant height or floral yield in this landrace. Theoretical models of mating-system evolution indicate that inbreeding depression arising from deleterious recessive mutations can constrain the evolution of self-fertilization (Lande and Schemske, 1985). Empirical and theoretical studies have suggested that inbreeding could facilitate the purging of deleterious recessive mutations, potentially reducing the magnitude of inbreeding depression over generations (Crnokrak and Barrett, 2002; Charlesworth and Willis, 2009). In hemp, inbreeding depression has been reported in certain genotypes (Kurtz et al., 2020); yet the magnitude of its expression appears to depend on genetic background and breeding history. The stable vegetative

performance and complete female expression observed in the selfed 'Hang Kra Rok' progenies may suggest that this landrace tolerates selfing without substantial fitness penalties in the short term, possibly due to historical selection and partial purging of deleterious alleles. The absence of detectable inbreeding depression in S_1 height and yield supports the hypothesis of historical purging of deleterious alleles, though future multi-generational studies are needed to confirm this genetic mechanism. Based on these findings, controlled self-pollination may enhance genetic uniformity, while maintaining short-term agronomic stability, which is desirable for commercial feminized seed production and standardized indoor cultivation systems.

Conclusion

Under the specific PFAL conditions evaluated, self-pollination was an effective strategy for producing 'Hang Kra Rok' feminized seeds, ensuring high sexual stability and earlier harvest in the evaluated accessions. It ensured 100% female plants and earlier flowering without reducing yield or cannabinoid quality. While cross-breeding increased plant height, it provided no significant benefits in potency or production efficiency.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

This research was funded by Kasetsart University, Bangkok, Thailand through the Graduate School Fellowship Program and the FOA Cannabis Research Project at the Vajiranusorn Building, Agricultural Research and Technology Transfer Center (ARTTC), Faculty of Agriculture, Kasetsart University. LABACTIVE CO., LTD., Bangkok, Thailand, provided the near-infrared spectroscopy equipment for cannabinoid analysis.

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