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SPEED BREEDING AS A PLATFORM FOR RAPID GENERATION TURNOVER AND RECESSIVE PHENOTYPE FIXATION IN *CANNABIS SATIVA* L.

SUBMITTED BY

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Introduction

Rapid climatic changes and strict market regulations necessitate accelerated crop improvement, particularly for industrial hemp (Cannabis sativa L.), which is naturally limited to a single generation per year. Traditional breeding methods are often too slow to stabilize essential traits such as specific cannabinoid profiles and fiber quality. Speed Breeding (SB), utilizing controlled environments to manipulate photoperiods and temperature, offers a solution by drastically shortening generation times; however, its application in short-day species like hemp remains underexplored.

This doctoral research validates the application of SB as a foundational platform for industrial hemp breeding. The primary objective was the rapid introgression and fixation of the recessive yellow-stem phenotype - valued for superior fiber processing - while simultaneously optimizing for high CBD and compliant low THC content. By managing the complex crossing of parents with divergent flowering times, the study aimed to condense years of breeding into a single calendar year. Additionally, the research evaluated the integration of modern drone-based phenotyping to ensure efficient selection. This dissertation documents the successful establishment of a robust SB methodology, resulting in a stabilized yellow-stemmed, high-CBD, and low-THC hemp line.

Objective of the study

The primary objective of this doctoral research was to innovate the breeding process for industrial hemp (Cannabis sativa L.) by integrating Speed Breeding (SB) technology with targeted genetic selection. This work aimed to establish a high-throughput platform capable of accelerating generation turnover and efficiently fixing multiple recessive traits, while simultaneously ensuring compliance with stringent regulatory standards.

Research Questions

1. Feasibility of Generation Acceleration: Can an integrated Speed Breeding (SB) protocol effectively shorten the life cycle of photoperiod-sensitive, dioecious Cannabis sativa lines - specifically those with highly divergent flowering times - to achieve and sustain a generation turnover of multiple cycles per year?

2. Trait Fixation and Inheritance: What is the most efficient breeding pathway (backcrossing vs. accelerated inbreeding) under SB conditions to fix the desirable recessive yellow-stem phenotype while simultaneously managing linked or associated undesirable traits, such as initial low seed yield and potential chemotype instability?

3. Phenotype-to-Chemotype Selection Efficacy: Can high-throughput, low-cost analytical methodologies (TLC) be reliably integrated into the accelerated breeding cycle for immediate selection and culling of plants to

maintain strict compliance with low-THC regulatory limits without critically compromising the concurrent selection for high CBD content and yield-related traits?

4. Environmental Predictability and Yield Stability: How do the highly controlled, supra-optimal thermal and photoperiod conditions imposed by the SB environment affect the subsequent field performance (maturity time, plant height, seed yield) of the advanced, fixed lines, and can a reliable method for phenological and morphological extrapolation be established?

Research Hypotheses

1. The application of a precisely controlled SB environment, utilizing customized photoperiod and thermal cycling, will significantly reduce the generation time of photoperiod-sensitive Cannabis sativa to achieve a minimum of four, and potentially up to six, generations annually, irrespective of the parental line maturity class.

2. The recessive yellow-stem phenotype can be stabilized and fixed in a homozygous state within 5 generations using a progeny-tested backcrossing strategy under SB conditions, successfully introgressing the trait from an early-maturing donor into diverse backgrounds without long-term genetic loss of vegetative vigor.

3. Rigorous selection based on high-throughput TLC screening for low-THC content will be sufficient to secure regulatory compliance in advanced lines, leading to populations where the CBD concentration stabilizes at a level significantly higher than the non-selected parental averages.

4. Advanced lines selected for compact architecture and high seed count under the high-density SB environment will demonstrate comparable or superior seed yield and Thousand-Kernel Weight (TKW) stability when subsequently validated in open-field conditions compared to their conventional parent cultivars.

Methodology

Experimental Site and Speed Breeding (SB) Platform

The research was conducted at an experimental greenhouse facility in Hédervár, Hungary. A speed breeding platform was established using 250 W full-spectrum LED fixtures with adjustable spectra and a blackout screen system to manipulate photoperiods.

Protocol: The seed-to-seed cycle was compressed to approximately 90 days. The photoperiodic regime included a long-day phase (16–24 hours) for vegetative growth, followed by a strict 12-hour dark period to induce synchronous flowering.

Cultivation: Plants were grown in 20-liter containers using a peat-clay substrate mixture, supported by an automated drip irrigation system delivering a phase-adjusted nutrient solution.

Plant Material and Breeding Strategy

The breeding program aimed to introgress the recessive yellow-stem phenotype into diverse genetic backgrounds while optimizing the cannabinoid profile (high CBD, low THC).

Parental Lines: The program utilized 'Chamaeleon' (yellow-stem donor) crossed with 'Balaton' (early-maturing) and 'Eletta Campana' (late-maturing, high-CBD).

Workflow: A family selection breeding method was applied, involving hybridization followed by serial backcrossing and family selection over eight accelerated generations.

Synchronization: Photoperiod manipulation was used to synchronize the flowering of early and late-maturing parental lines to enable successful hybridization.

Measurements and Evaluations

Morphological Characterization: Phenotypic data were recorded according to the Community Plant Variety Office (CPVO) protocols, distinguishing traits such as stem colour, plant height, and flowering time. Data entry was streamlined using a custom Android-based digital framework with barcode traceability.

Chemical Analysis: A high-throughput selection strategy was employed using Thin-Layer Chromatography (TLC) to screen thousands of individuals for cannabinoid content (THC/CBD). Confirmatory analyses were conducted using High-Performance Liquid Chromatography (HPLC).

High-Throughput Phenotyping: Unmanned Aerial Vehicle (UAV) imagery was utilized to calculate the Visible Atmospherically Resistant Index (VARI) for assessing field performance and leaf colour.

Data Analysis Statistical evaluations, including ANOVA and Principal Component Analysis (PCA), were performed using R software and ARM 9.1.0 to assess trait stability and genetic gain across cycles.

Result and Discussion

The study successfully validated a Speed Breeding platform for industrial hemp, achieving four generations per year (approx. 90-day cycles) regardless of the initial flowering time of the parental lines.

The accelerated cycles maintained high seed germination rates of over 80% in advanced lines and did not compromise Thousand-Kernel Weight, which showed a progressive increase in the early-maturing selection line.

Regarding trait inheritance, initial F1 crosses yielded only green-stemmed plants, confirming the recessive nature of the yellow-stem trait. Through serial backcrossing and rigorous family selection, this trait was completely fixed to 100% homozygosity by the fifth generation in both early- and medium-maturing lines. In terms of chemical profile, a clear linear trend of increasing CBD content ($r = 0.993$) was achieved in the medium-maturing line over successive cycles, while strict selection pressure ensured THC levels remained below the legal limit of 0.2%. Additionally, the use of UAV-based remote sensing was validated, showing strong correlations with manual measurements for plant height ($r=0.93$) and chlorophyll content ($r=0.76$).

By manipulating photoperiods to include 12 hours of strict darkness, the highly divergent flowering times of early and late parents were successfully synchronized to enable hybridization. While the yellow-stem trait was successfully introgressed, segregation in F2 and backcross generations deviated slightly from strict Mendelian ratios, suggesting incomplete dominance or environmental influence which necessitated a light green classification class.

Early generations revealed an unfavourable linkage where the yellow-stem phenotype was associated with lower CBD and seed yield, but this was successfully broken through multi-cycle selection. While High-throughput Thin-Layer Chromatography was effective for rapid, low-cost culling, its tendency to overestimate THC content likely slowed maximal CBD gain, indicating a need for balanced screening methods. Furthermore, while qualitative traits like stem colour transferred perfectly to the field, quantitative traits like final plant height and maturity time in the late-maturing line were harder to predict solely from greenhouse data due to the drastically reduced vegetative phase in the Speed Breeding environment.

Conclusion

The research successfully established and validated a novel Speed Breeding platform tailored for industrial hemp. By precisely controlling photoperiod, temperature, and environmental conditions, the system achieved a consistent seed-to-seed cycle of approximately 90 days, enabling eight selection cycles over two years. This platform effectively overcame the species' primary limitations of dioecy and photoperiod sensitivity, demonstrating that rapid generation turnover is feasible without compromising seed viability or yield.

The efficacy of the platform was proven through the targeted introgression and fixation of the recessive yellow-stem trait and the effective management of chemotypes to achieve superior CBD:THC ratios. Advanced lines maintained high agronomic quality, demonstrating that speed breeding does not necessitate a trade-off between speed and quality. Ultimately, the research demonstrates that Speed Breeding is a robust, scalable platform that reduces the breeding timeline from over eight years to just two, providing a confirmed pathway for developing legally compliant and agronomically superior hemp cultivars.

As a conclusion the research demonstrates that Speed Breeding is a robust, scalable platform for industrial hemp. It allows for the rapid fixation of qualitative traits (yellow stem) and the efficient management of chemotypes (low THC) within a compressed timeframe (2 years vs. 8+ years), providing a confirmed pathway for developing legally compliant, agronomically superior hemp cultivars.

Novel Scientific Results of Doctoral Research

Validation of a Cannabis sativa Speed Breeding (SB) Platform for Regulatory Compliance

Demonstration and validation of an integrated SB system that overcomes the asynchronous flowering of genetically diverse Cannabis parental lines (early-maturing×late-maturing) to successfully achieve eight generational cycles over two years while maintaining seed viability and yield stability.

Optimalization of a Cannabis sativa Speed Breeding (SB) Platform for Genetic Gain

Quantification of genetic gain in Cannabidiol (CBD) content under severe selection pressure, documenting a clear linear trend ($r = 0.993$) across the accelerated cycles of the 'E' line, confirming that significant chemotype progress can be achieved concurrently with rigorous regulatory culling.

Characterization of Unfavourable Genetic Linkage in Industrial Hemp Germplasm

Identification and characterization of an unfavourable genetic association or pleiotropic effect in early-generation heterozygotes derived from the Chamaeleon cross, where the desired yellow-stem phenotype was linked to reduced CBD content and weak seed yield. This novel observation highlights a critical genetic constraint for multi-trait breeding strategies involving the industrial yellow-stem trait.

Documentation of Minor Cannabinoid Genetic Potential

Documentation of the genetic potential for minor cannabinoid biosynthesis (specifically Cannabichromene, CBC) in the early generations of novel industrial hemp lines (e.g., 0.143% CBC in Cycle-1-B), demonstrating the presence of this valuable genetic background despite its subsequent loss due to a breeding focus solely on the major CBD:THC chemotype.

Publications

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