Agricultural Sciences, 2022, 13, 1291-1308
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Cloning Successive Generations of Industrial Hemp (*Cannabis sativa*) to Assess Cannabinoid Profiles

Cassandra Perrone[1], Paul Kline[2,3], John DuBois[1,3]

[1]Department of Biology, Middle Tennessee State University, Murfreesboro, USA
[2]Department of Chemistry, Middle Tennessee State University, Murfreesboro, USA
[3]Tennessee Center for Botanical Medicine Research, Middle Tennessee State University, Murfreesboro, USA
Email: John.dubois@mtsu.edu

How to cite this paper: Perrone, C., Kline, P. and DuBois, J. (2022) Cloning Successive Generations of Industrial Hemp (*Cannabis sativa*) to Assess Cannabinoid Profiles. *Agricultural Sciences*, **13**, 1291-1308.
https://doi.org/10.4236/as.2022.1312079

Received: November 3, 2022
Accepted: December 5, 2022
Published: December 8, 2022

Abstract

Industrial hemp (*Cannabis sativa*) has made a remarkable impact worldwide due to the plant's beneficial properties and versatile use. Vegetative cuttings (clones) of *C. sativa* are the preferred propagation technique to be the most effective in retaining the same genetic information and reducing hybridization and mutations. The objective of this project was to assess cannabinoid profile concentrations of successively cloned generations of 5 varieties: Cherry, Cherry Blossom, Cherry × Workhorse, Sour Space Candy, and The Wife. This research project focused on the idea that every cloned plant contains the exact same genetic information and, therefore, should have the same metabolic profile of cannabinoids through all the successive generations grown, which is shown to be true. Plants were cloned for multiple generations using stem cuttings and a commercial cloner. As plants matured, they were set in environmental conditions to stimulate flowering and buds were harvested and analyzed for cannabinoid contents using HPLC. Several generations of each variety were successfully cloned. As many as 17 different cannabinoids were analyzed and the results of this study show that there is not a significant difference in cannabinoids over successive generations, showing no major trends.

Keywords

Industrial Hemp, Cannabis, Cloning, Cannabinoids

1. Introduction

Industrial hemp (*Cannabis sativa*) has made a significant impact across the globe

due to the plant's beneficial properties and versatile use. Over the past decade, there has been an increase in research studies on *C. sativa* and how this single plant can revolutionize the pharmaceutical and agricultural industries [1]. *Cannabis sativa* contains many different chemical components, but two substances of interest are Δ9- tetrahydrocannabinol (THC) and Cannabidiol (CBD), which are both cannabinoids. Cannabinoids are produced as secondary metabolites. Therefore, these compounds are produced to help the plant thrive in the environment but are not necessary to live [2].

Besides pharmaceutical usage of the plant, *C. sativa* has been known to be used for its natural hemp fibers to produce various goods such as carpets and ropes [3]. Hemp fibers attain an extremely high tensile strength (300 - 800 MPa)—high resistance to breaking under tension—which allows hemp fibers to be a substitution for synthetic fibers in polymer composite reinforcement [3]. Thus, the various properties of *C. sativa* have been accessed globally for new medical and materialistic applications.

Vegetative cloning is the process of taking a portion (cutting) of a plant to asexually grow an entirely new plant [4]. To retain cannabinoid production levels throughout multiple generations, vegetative cutting is the most effective propagation technique. Research has shown that cannabinoid profiles change due to different genetics and mutations as generations are grown via seeds [5]. In addition, the levels of the cannabinoids were over 4.1× greater in the plants grown vegetatively rather than those that grew from seeds [5]. Additionally, as plants are cloned, a mutation, commonly known as a loss-of-function gene, gets "activated", in other words, the plants typically lose a function over multiple generations of vegetative cloning [6]. After observing multiple clonal generations, the plants themselves become less robust as the generations continue [6]. Also, the plants that the cuttings are taken from are more prone to diseases as well as harmful insects [6].

To our knowledge, there has been no study that assesses the cannabinoid contents in Industrial hemp over successively cloned generations. This research project revolved around the idea that all cloned plants have the same genetic information—metabolic production of cannabinoids should remain consistent throughout successive generations. The goals for this study were 1) successfully growing successive clonal generations from vegetative cuttings and 2) harvesting buds and analyzing cannabinoid levels to see if the function of producing cannabinoids is lost.

2. Methods

Five different varieties of *C. sativa* were used in this project, specifically: The Wife, Sour Space Candy, Cherry, Cherry Blossom, and Cherry × Workhorse. All the plants in this research were female. Cuttings were obtained for a new generation by the inspection of each plant (of the same variety and generation) for optimal stems that contained at least 3 nodes and were approximately 8 cm long to

be placed in the cloner (Botanicare, Model RESLPWHB-40). In addition, a new razorblade was used to ensure the cut was clean as well as at an angle to achieve the maximum surface area possible on the stem. The leaves were clipped using shears to reduce the loss of water while in the cloner. Each freshly cut stem had been coated with a rooting gel, Clonex, and then placed in a cloner to obtain healthy root growth.

The cloner was set to a 16 h:8 h ratio of light:dark schedule to guarantee that the cuttings remain in a vegetative state to prevent the process of flowering. This process normally took around 2 - 3 weeks for adequate root growth, and once it was achieved, the plants from which the cuttings were taken were moved into a growth chamber.

The new generation of plants (rooted cuttings) had been potted in 8 cm pots with MiracleGro potting soil to proceed growing to around 15 cm tall. At around 15 cm of growth, each plant was moved into a 15 cm pot. The plants were allowed to mature to around 1/2 to 1 m tall in the greenhouse. As these plants were maturing, cuttings for the next generation were taken and placed in the cloner.

After the plants reached the desired height and the next generation cuttings were rooting properly, they were placed in growth chambers set at 8 h:16 h light:dark to stimulate flowering. The growth chambers were 1.2 m × 2.4 m × 1.8 m tall with LED growth lights to help the plants induce flowering over a 3 - 4-week period. Once flowering had occurred, buds were harvested and air dried for two weeks before they were analyzed for cannabinoid content following the procedures developed in our laboratory [7]. High-Performance Liquid Chromatography (HPLC) was used to analyze the cannabinoid profiles of each sample.

HPLC analysis is typically used for cannabinoid analysis [8]. Preparation for HPLC was initiated by taking 100 mg of air-dried bud sample (each generation for the varieties was completed individually) and placing it in a 50 mL centrifuge tube. Exactly 25 mL of 95% ethanol was placed in the 50 mL centrifuge tube. The goal was to have 10 plants per generation per variety (five samples made per plant). All samples were vortexed for 1 minute on speed level 10 and then placed in the centrifuge; the samples were centrifuged for 2 minutes at 4˚ C, and at 2000 RPM. Syringes were prepared by the attachment of a Millex HV 0.45 μm Filter to the opening (each sample had its own syringe and filter to prevent contamination). Each sample had 1 mL of solution extracted which was placed in a 1.5 mL vial, labeled, capped, and then stored in a cold refrigerated room until they were analyzed.

The HPLC system was the Dionex UltiMate 3000 and the specific column was the Phenomenex Kinetex EVO 5 μm C18 100 Å (150 × 4.6 mm) column. The mobile phases that eluted the cannabinoids consisted of methanol with 0.1% formic acid (B) and water with 0.1% formic acid (A). Additionally, the flow rate and temperature were 1.0 mL/minute and 50 degrees Celsius. The eluent method used for the result is a linear gradient which after 45 minutes was 60% B/40% A

to 95% B/5% A. Every hour the HPLC system graphed the cannabinoid level concentrations and took in a new sample to analyze.

The cannabinoid level concentrations were compared to standards through their individual Peak Area Retention Time, and clonal generation cannabinoid levels were compared through ANOVA (Analysis of Variance). This process was used on each successive generation for all five varieties of *C. sativa* to compare the cannabinoid profiles.

3. Results

Cherry—Ten successive generations of Cherry were cloned, flowered, harvested, and analyzed by HPLC. All cannabinoids were analyzed via ANOVA with alpha = 0.05. Every generation that had detectable results had an F calculated value less than the F critical value; thus, there is no significant difference between successive generations of Cherry. The cannabinoid CBDVA remained statistically consistent throughout successive generations (**Figure 1**). Also following this trend was the cannabinoid CBDA; the data showed consistency across successive generations (**Figure 2**). The cannabinoid Δ9THC showed a rapid decrease across successive cloned generations, but the decrease had not been significant enough to make this cannabinoid have an F calculated value larger than the F critical value (**Figure 3**). The cannabinoids CBDV, CBD, and CBG analyzed in Cherry showed no trend due to experiment error because the data did not stay consistent, increase, or decrease (**Figures 4-6**). There had been a slight trend for the cannabinoid CBL. For CBL, the production had stopped after generation 2; there was not considered a difference in this cannabinoid because there was only a small concentration before the production stopped (**Figure 7**).

Cherry Blossom—Ten successive generations of Cherry Blossom were cloned, flowered, harvested, and analyzed (Generations 8 and 9 not analyzed) by HPLC. Two cannabinoids that were not present were CBDV and CBG. All cannabinoids were analyzed via ANOVA with alpha = 0.05. Every generation that had detectable results had an F calculated value less than the F critical value; this means that we fail to reject the null hypothesis that there is no difference between successively cloned generations of cannabinoid % mass values. Thus, there is no difference between successive generations of Cherry Blossom. The cannabinoid CBDVA has remained consistent throughout successive generations (**Figure 8**). All the other cannabinoids analyzed for Cherry Blossom (besides CBL) showed no trend due to experimental error; this is because the cannabinoids did not remain consistent, increase, or decrease over time (**Figures 9-11**). The cannabinoid CBL had stopped being produced after generation 3; there was not considered a difference in this cannabinoid because there was only a small concentration before the production stopped (**Figure 12**).

Cherry × Workhorse—Ten successive generations of Cherry × Workhorse were cloned, flowered, harvested, and analyzed (generation 7 not analyzed) by

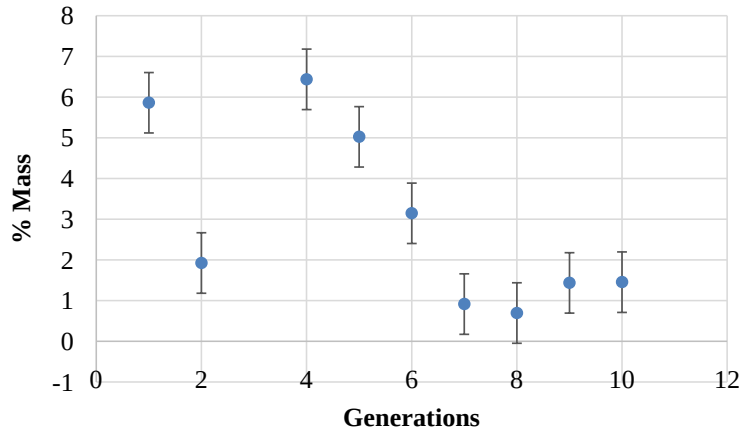

Figure 1. CBDVA % mass in Cherry variety (mean ± SE). The p value was 0.98696. The F calculated value was 0.18433 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDVA.

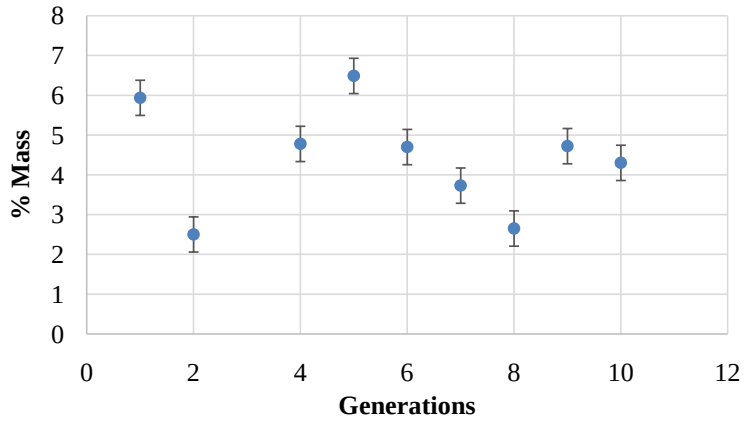

Figure 2. CBDA % mass in Cherry variety (mean ± SE). The p value was 0.66941. The F calculated value was 0.72507 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDA.

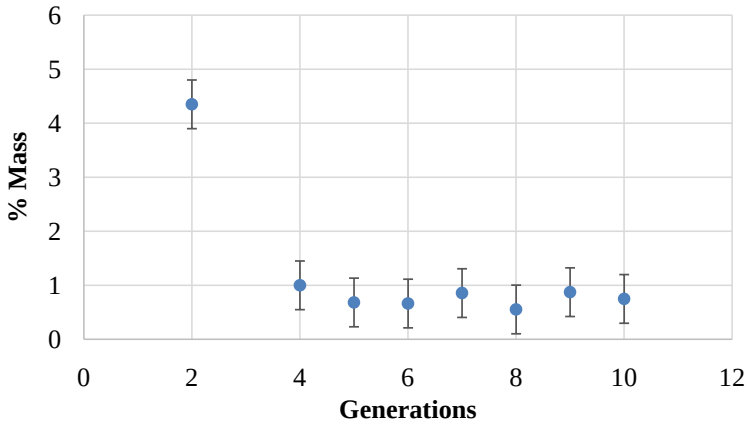

Figure 3. Δ9-THC % mass in Cherry variety (mean ± SE). The p value was 0.9959. The F calculated value was 0.10787 and the F critical value was 3.50046; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid Δ9-THC.

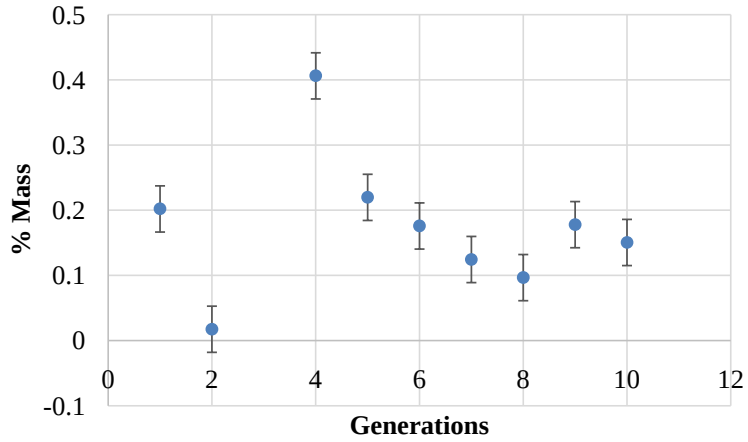

Figure 4. CBDV % mass in Cherry variety (mean ± SE). The p value was 0.97317. The F calculated value was 0.23522 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDV.

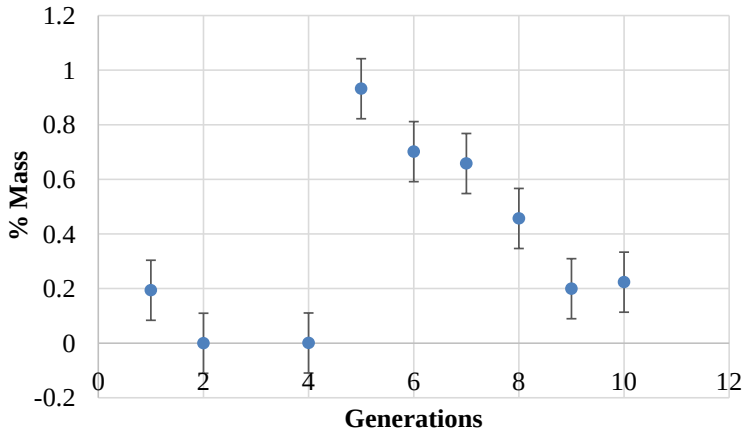

Figure 5. CBD % mass in Cherry variety (mean ± SE). The p value was 0.96093. The F calculated value was 0.26916 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBD.

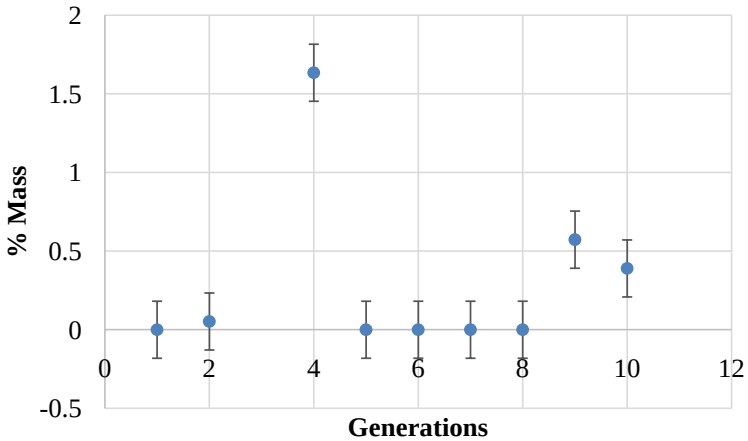

Figure 6. CBG % mass in Cherry variety (mean ± SE). The p value was 0.96647. The F calculated value was 0.254613 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBG.

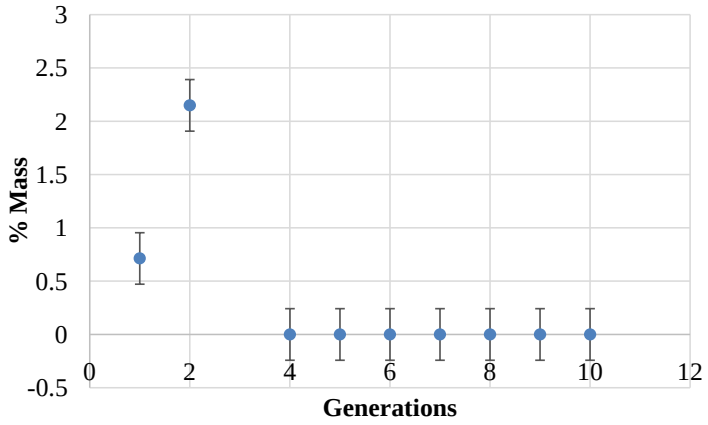

Figure 7. CBL % mass in Cherry variety (mean ± SE). The p value was 0.98948. The F calculated value was 0.17195 and the F critical value was 3.22958; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBL.

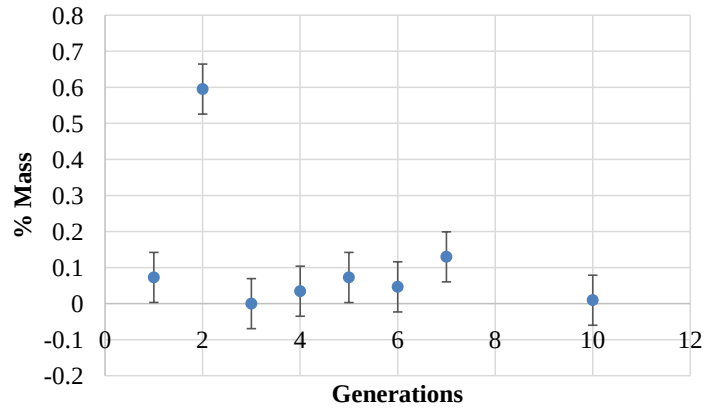

Figure 8. CBDVA % mass in Cherry Blossom variety (mean ± SE). The p value was 0.0.687111. The F calculated value was 0.715257 and the F critical value was 3.38813; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDVA.

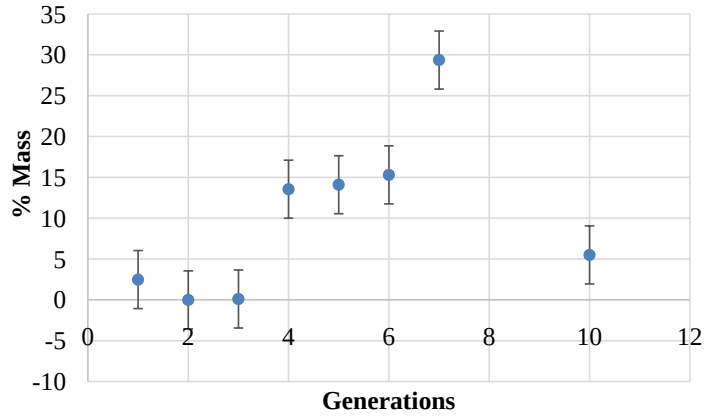

Figure 9. CBDA % mass in Cherry Blossom variety (mean ± SE). The p value was 0.771503. The F calculated value was 0.59637 and the F critical value was 3.38813; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDA.

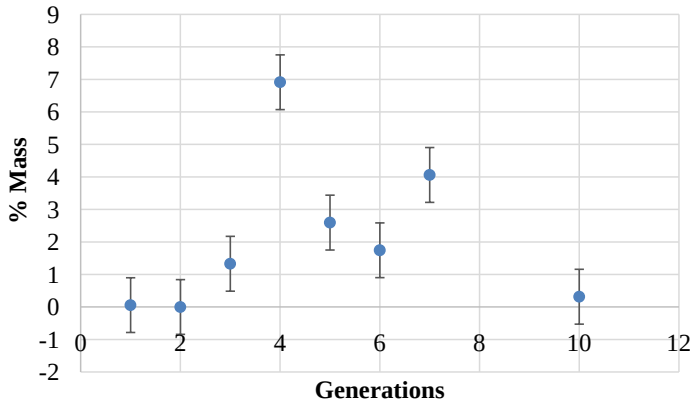

Figure 10. CBD % mass in Cherry Blossom variety (mean ± SE). The p value was 0.374408. The F calculated value was 1.267546 and the F critical value was 3.38813; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBD.

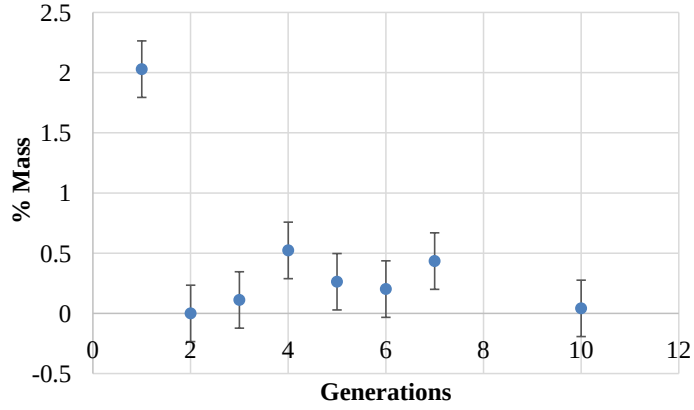

Figure 11. Δ9-THC % mass in Cherry Blossom variety (mean ± SE). The p value was 0.74228. The F calculated value was 0.690901 and the F critical value was 3.38813; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid Δ9-THC.

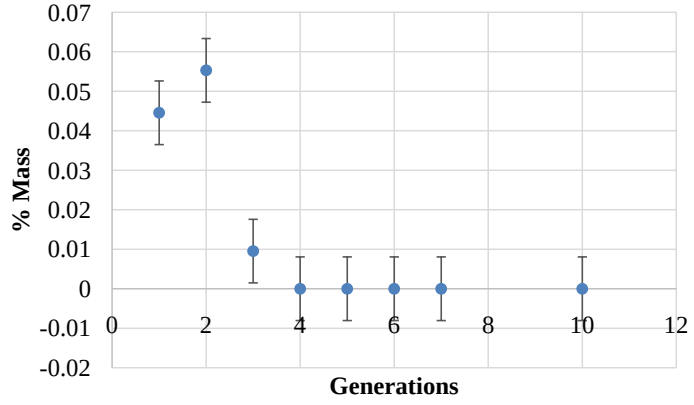

Figure 12. CBL % mass in Cherry Blossom variety (mean ± SE). The p value was 0.686863. The F calculated value was 0.715611 and the F critical value was 3.38813; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBL.

HPLC. All cannabinoids were analyzed via ANOVA with alpha = 0.05. Every generation that had detectable results had an F calculated value less than the F critical value; this means that we fail to reject the null hypothesis that there is no difference between successively cloned generations of cannabinoid % mass values. Thus, there is no difference between successive generations of Cherry × Workhorse. The cannabinoid CBDVA had remained consistent throughout all analyzed generations (Figure 13). Likewise, the cannabinoid CBDV had remained consistent throughout all analyzed generations (Figure 14). The randomness for the following cannabinoids can be explained by experimental error: CBDA, CBD, Δ9-THC, and CBG (Figures 15-18). These cannabinoids showed no trend over being cloned successively. The cannabinoid CBL shows a decrease in % mass levels over time and has stopped being produced after generation 3; there was not considered a difference in this cannabinoid because there was only a small concentration before the production stopped (Figure 19).

Sour Space Candy—Seven successive generations of Sour Space Candy were cloned, flowered, harvested, and analyzed (generation 5 not analyzed) by HPLC. There was one cannabinoid that was not detected in every successive generation: CBG. All cannabinoids were analyzed via ANOVA with alpha = 0.05. Every generation that had detectable results had an F calculated value less than the F critical value; this means that we fail to reject the null hypothesis that there is no difference between successively cloned generations of cannabinoid % mass values. Thus, there is no difference between successive generations of the same cannabinoid in Sour Space Candy. There is no trend for CBDVA, CBDV, CBDA, CBD, and Δ9-THC analyzed for Sour Space Candy as the % mass levels do not remain consistent, increase, or decrease (Figures 20-24). The cannabinoid CBL stopped being produced after generation 3; there was not considered a difference in this cannabinoid because there was only a small concentration before the production stopped (Figure 25).

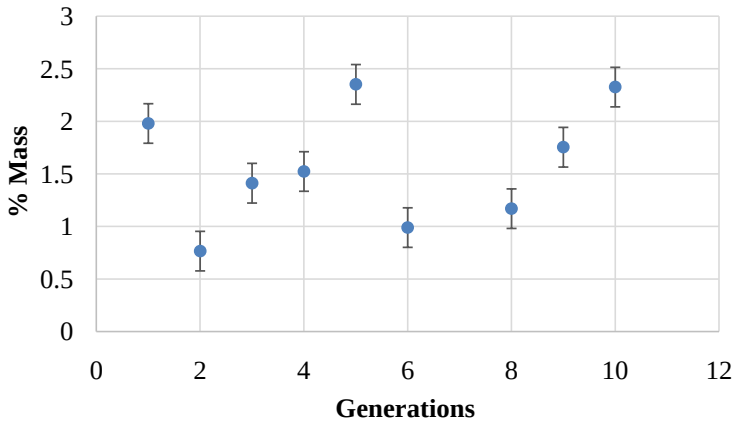

Figure 13. CBDVA % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.79526. The F calculated value was 0.566098 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDVA.

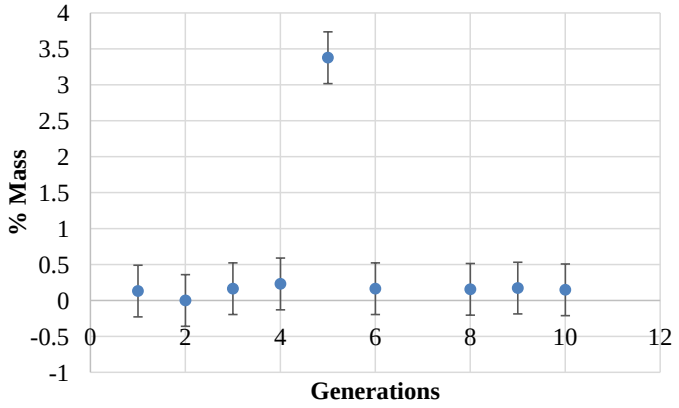

Figure 14. CBDV % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.90633. The F calculated value was 0.399044 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDV.

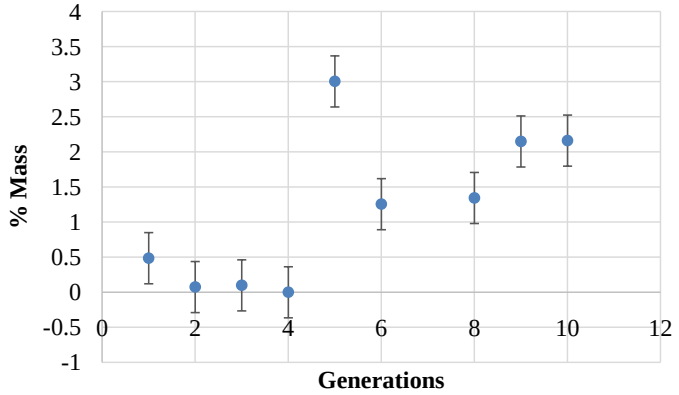

Figure 15. CBDA % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.669192. The F calculated value was 0.74042 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDA.

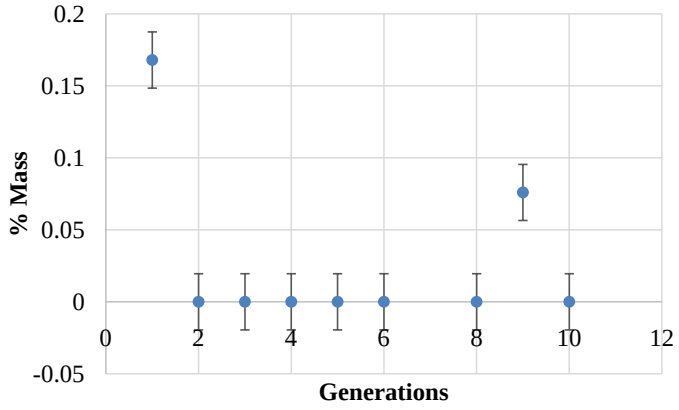

Figure 16. CBD % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.93692. The F calculated value was 0.342431 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBD.

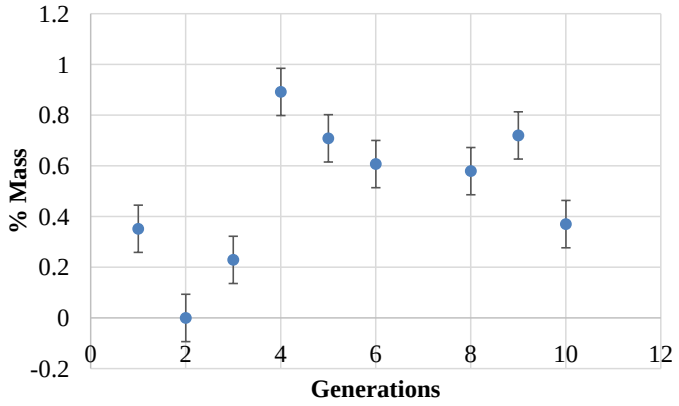

Figure 17. Δ9-THC % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.898164. The F calculated value was 0.412846 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid Δ9-THC.

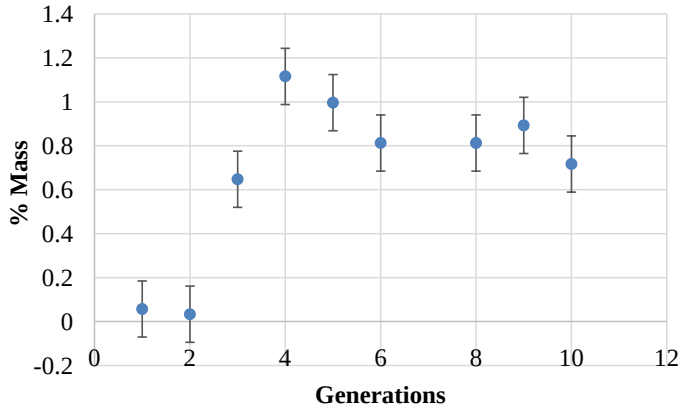

Figure 18. CBG % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.86668. The F calculated value was 0.462864 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBG.

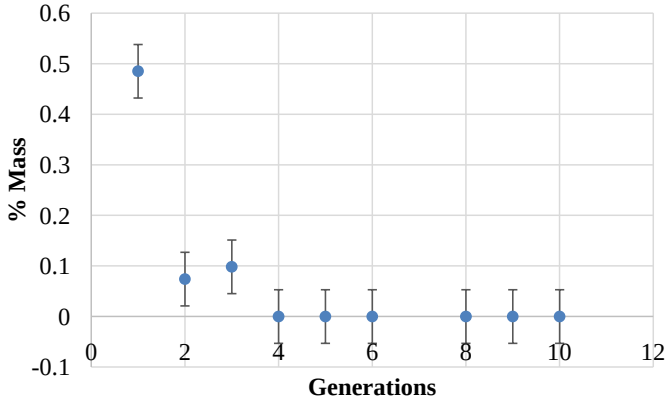

Figure 19. CBL % mass in Cherry × Workhorse variety (mean ± SE). The p value was 0.943388. The F calculated value was 0.329032 and the F critical value was 3.178893; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBL.

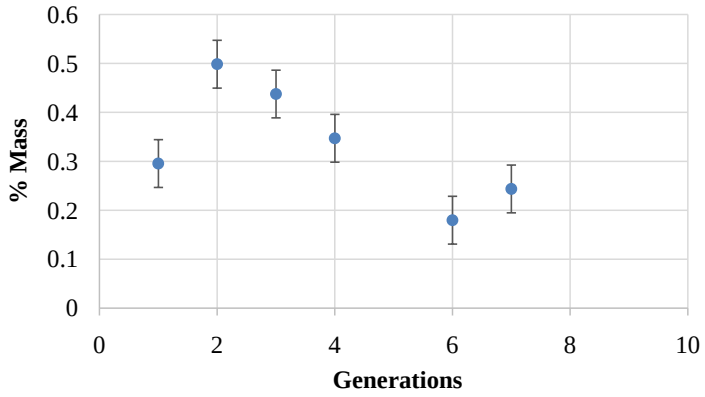

Figure 20. CBDVA % mass in Sour Space Candy variety (mean ± SE). The p value was 0.8556766. The F calculated value was 0.3994066 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDVA.

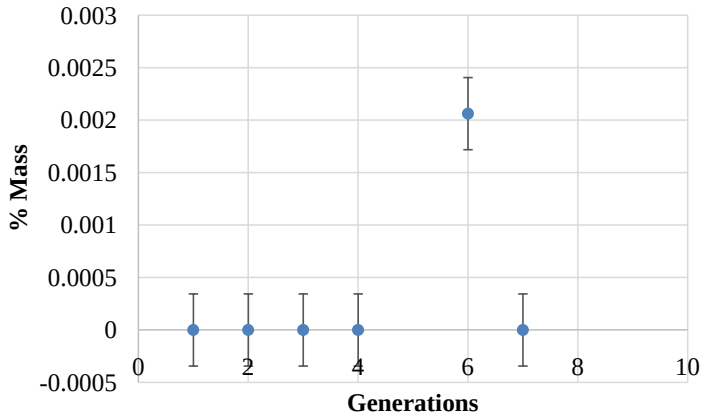

Figure 21. CBDV % mass in Sour Space Candy variety (mean ± SE). The p value was 0.8641148. The F calculated value was 0.3860969 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDV.

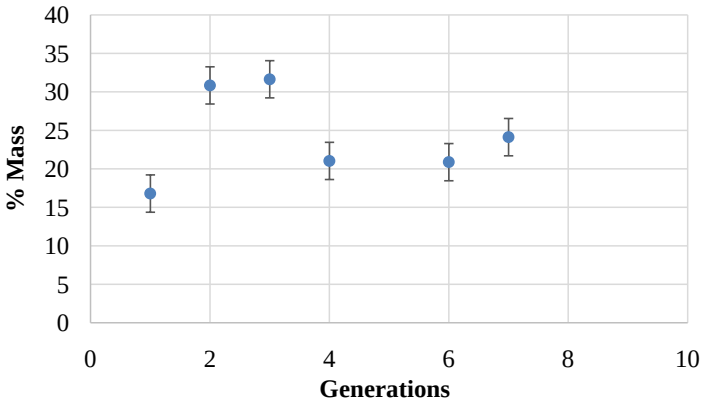

Figure 22. CBDA % mass in Sour Space Candy variety (mean ± SE). The p value was 0.9881642. The F calculated value was 0.1263014 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDA.

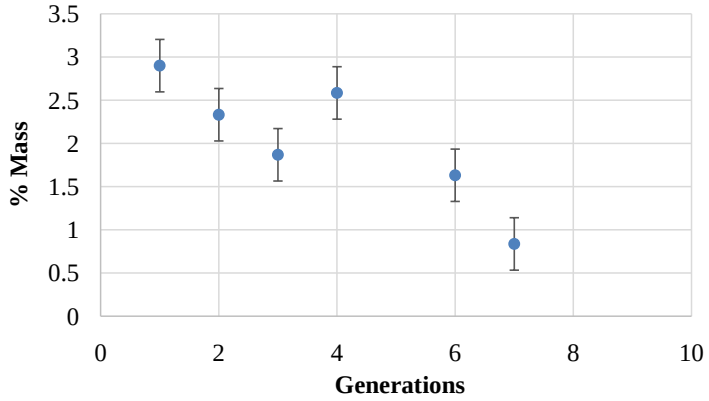

Figure 23. CBD % mass in Sour Space Candy variety (mean ± SE). The p value was 0.884422. The F calculated value was 0.3533805 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBD.

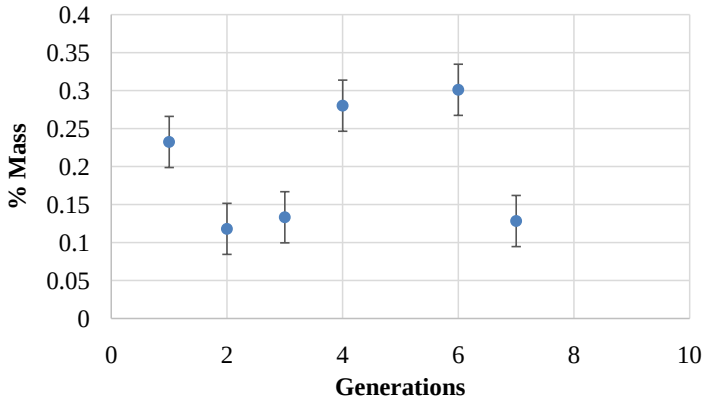

Figure 24. Δ9-THC % mass in Sour Space Candy variety (mean ± SE). The p value was 0.8480999. The F calculated value was 0.4112489 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid Δ9-THC.

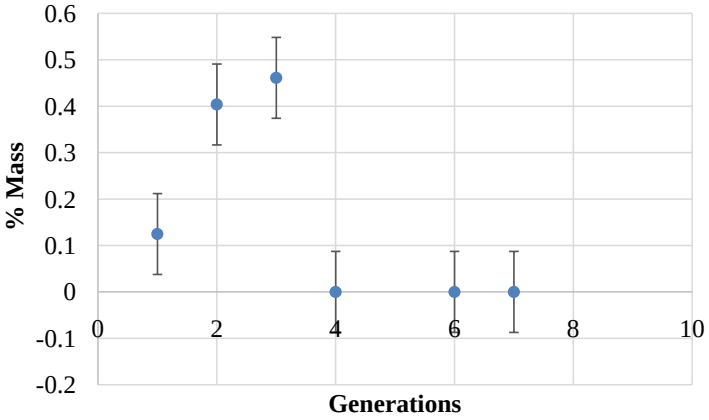

Figure 25. CBL % mass in Sour Space Candy variety (mean ± SE). The p value was 0.8756019. The F calculated value was 0.367727 and the F critical value was 4.2838657; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBL.

The Wife—Six Six successive generations of The Wife were cloned, flowered, harvested, and analyzed by HPLC. Two cannabinoids that were not detected were CBDV and CBG. All cannabinoids were analyzed via ANOVA with alpha = 0.05. Every generation that had detectable results had an F calculated value less than the F critical value; this means that we fail to reject the null hypothesis that there is no difference between successively cloned generations of cannabinoid % mass values. Thus, there is no difference between successive generations of The Wife. All the cannabinoids except CBL showed no trend as the % mass levels did not remain consistent, increase, or decrease (Figures 26-29). The cannabinoid CBL had stopped being produced after generation 2; there was not considered a difference in this cannabinoid because there was only a small concentration before the production stopped (Figure 30).

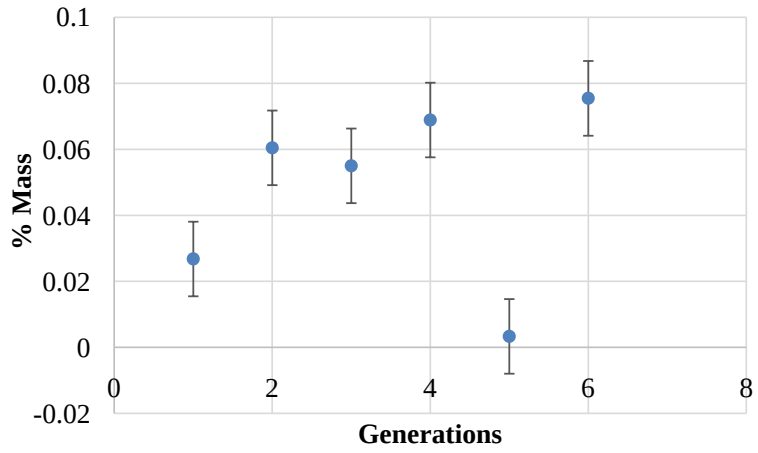

Figure 26. CBDVA % mass in The Wife variety (mean ± SE). The p value was 0.931962. The F calculated value was 0.237425 and the F critical value was 4.387374; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDVA.

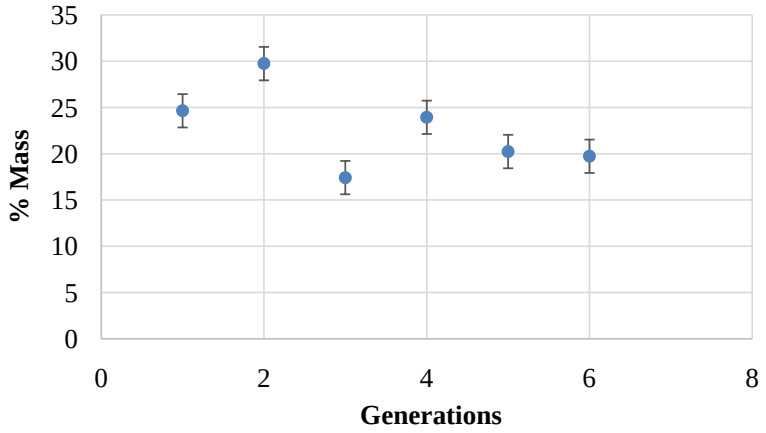

Figure 27. CBDA % mass in The Wife variety (mean ± SE). The p value was 0.998982. The F calculated value was 0.034936 and the F critical value was 4.387374; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBDA.

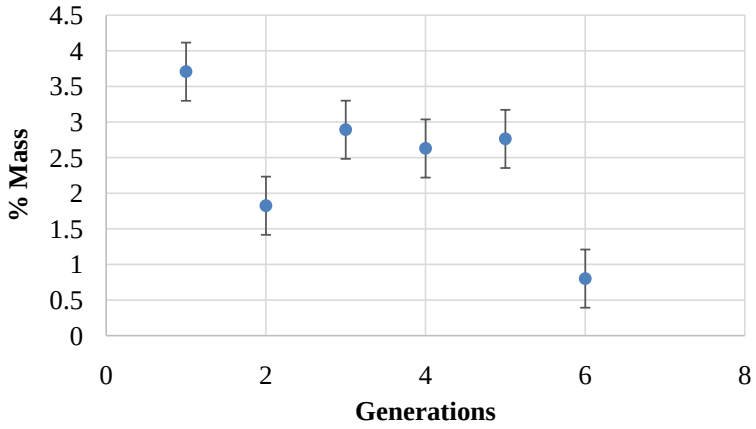

Figure 28. CBD % mass in The Wife variety (mean ± SE). The p value was 0.892414. The F calculated value was 0.30635 and the F critical value was 4.387374; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBD.

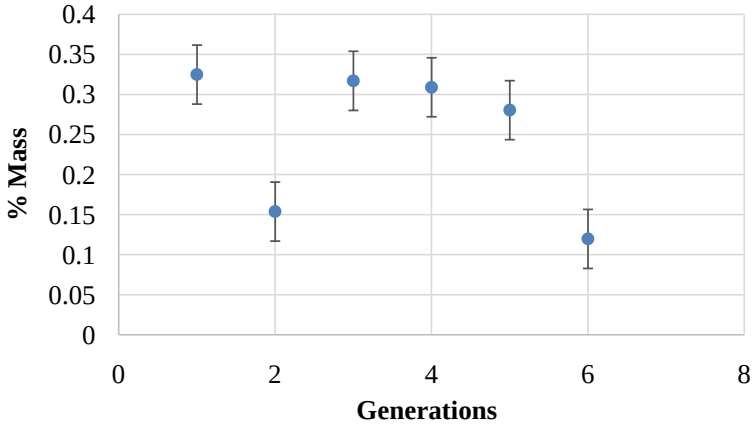

Figure 29. Δ9-THC % mass in The Wife variety (mean ± SE). The p value was 0.653994. The F calculated value was 0.6873563 and the F critical value was 4.387374; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid Δ9-THC.

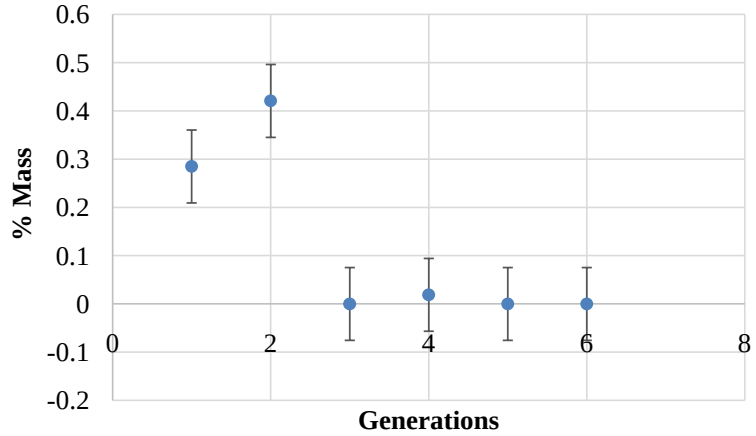

Figure 30. CBL % mass in The Wife variety (mean ± SE). The p value was 0.949703. The F calculated value was 0.202624 and the F critical value was 4.387374; there is no statistical difference between % mass in successive generations of *C. sativa* for the cannabinoid CBL.

4. Discussion

A horticulture study on cloning [9] stated that morphological changes, such as the growth of male organs, had occurred after the seventh successive generation. This study indicated that the mutations occurred due to the nucleotide sequences in the genome of the cuttings taken for the new plant [9]. Therefore, those mutations will continue in the next successive generations, which alter the metabolic profile of *C. sativa*, which affects the overall % mass of cannabinoids in the plant.

The results of our study show that there is not a significant difference in cannabinoids over successive generations, although the % mass levels were variable but with no trends. The only cannabinoid to show a slight decrease over successive generations was Δ9-THC in the variety Cherry (**Figure 3**). All the generations of Sour Space Candy had high CBDA % mass levels of around 30%; Sour Space Candy is known to have high CBDA/CBD levels (CBDA decarboxylates into CBD), so each successive generation grown exhibited this trend (**Figure 22**). An objective of this research was to observe if the plants lose the function of producing cannabinoids overtime which had been shown in some cannabinoids: this occurred for CBL in every variety grown; there was not considered a difference in this cannabinoid in any variety because there was only a small concentration produced before the production stopped completely (**Figure 7**, **Figure 12**, **Figure 19**, **Figure 25**, **Figure 30**). CBDV and CBG had not been produced in any successive generation in the following varieties: The Wife and Cherry Blossom. The idea that all the cloned plants should have the same metabolism throughout multiple successive generations has been shown with these data for every variety.

According to Punja *et al.* [10], *C. sativa* is naturally a dioecious (female and male flowers are on separate plants) species but can turn to a monoecious (female and male flowers on the same plant) species spontaneously or under certain physical/chemical conditions. Female plants that undergo environmental stressors such as late harvest, changes in photoperiod, non-ideal temperatures, or hormone additives can cause male organs to grow. If the plants had been placed in the flowering chamber too early in development to flower, the extended dark period specifically triggers this formation [10]. From generation 5 onward, for the variety Cherry × Workhorse, the plants had all reverted to hermaphroditism (**Figure 31**). Generations 5 and 6 had been in the chamber together. When the plant buds were harvested and dried, seeds were found in the buds of the generation 6 plants. These seeds had then been germinated and planted to show whether the seeds were viable, which they were. All the successfully germinated seeds had produced healthy plants which happened to be all female. The amount of pollen produced by hermaphroditic plants is known to be significantly less in quantity than pollen produced by male plants [8]. Therefore, this allows for the assumption that the hermaphrodites still carried a XX genotype regardless of the flowers present in the monoecious plant and had viable pollen [11].

Figure 31. Generation 5 of Cherry × Workhorse reverted to hermaphroditism. Pistil (female) and Stamen (male) organs are indicated with arrows.

This research is beneficial for the future propagation of *C. sativa* as it shows that there is no significant trend (increase or decrease) in cannabinoid levels over successive generations. For legal purposes, *C. sativa* plants must maintain a THC concentration below 0.3% for the plants to be considered industrial hemp [12]. Thus, *C. sativa* growers need to be extremely aware of the THC concentrations in the plants because of the lack of stable cannabinoid levels. This shows that THC levels do not increase over successively cloned generations. Therefore, clonal propagation of *C. sativa* is an efficient method without affecting cannabinoid levels.

Acknowledgements

Funding for this study was provided by GreenWay Herbal Products, LLC (Murfreesboro, TN, USA) and a grant for the MTSU Undergraduate Research Experience and Creative Activity (URECA) program (CP-PI).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bilalis, D., Karidogianni, S., Roussis, I., Kouneli, V., Kakabouki, I. and Folina, A. (2019) *Cannabis sativa* L.: A New Promising Crop for Medical and Industrial Use. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*: *Horticulture*, **76**, 145-150. https://doaj.org/article/12e4db4130564b1ea4abaf27c88a4b99 https://doi.org/10.15835/buasvmcn-hort:2019.0020

[2] Gonçalves, J., Rosado, T., Soares, S., Simão, A.Y., Caramelo, D., Luís, A., Fernández, N., Barroso, M., Gallardo, E. and Duarte, A.P. (2019) Cannabis and Its Secondary Metabolites: Their Use as Therapeutic Drugs, Toxicological Aspects, and Analytical

Determination. *Medicines*, **6**, Article 31. https://doi.org/10.3390/medicines6010031

[3] Behera, S., Gautam, R.K., Mohan, S. and Chattopadhyay, A. (2021) Hemp Fiber Surface Modification: Its Effect on Mechanical and Tribological Properties of Hemp Fiber Reinforced Epoxy Composites. *Polymer Composites*, **42**, 5223-5236. https://doi.org/10.1002/pc.26217

[4] Priestley, J.H. and Swingle, C.F. (1929) Vegetative Propagation from the Standpoint of Plant Anatomy. Department of Agriculture, Washington DC. https://hdl.handle.net/2027/uc1.aa0007233257

[5] Coffman, C.B. and Gentner, W.A. (1979) Greenhouse Propagation of *Cannabis sativa* L. by Vegetative Cuttings. *Economic Botany*, **33**, 124-127. https://link.springer.com/article/10.1007/BF02858280 https://doi.org/10.1007/BF02858280

[6] Adhikary, D., Kulkarni, M., El-Mezawy, A., Mobini, S., Elhiti, M., Gjuric, R., Ray, A., Polowick, P., Slaski, J.J., Jones, M.P. and Bhowmik, P. (2021) Medical Cannabis and Industrial Hemp Tissue Culture: Present Status and Future Potential. *Frontiers in Plant Science*, **12**, Article 627240. https://doi.org/10.3389/fpls.2021.627240

[7] Shutes, K. (2019) Initiation in Cannabinoid Assessment of Trichomes on Industrial Hemp: (*Cannabis sativa*) Tissue Callus. Middle Tennessee State University, Murfreesboro.

[8] De Backer, B., Debrus, B., Lebrun, P., Theunis, L., Dubois, N., Decock, L., Verstraete, A., Hubert, P. and Charlier, C. (2009) Innovative Development and Validation of an HPLC/DAD Method for the Qualitative and Quantitative Determination of Major Cannabinoids in Cannabis Plant Material. *Journal of Chromatography B*, **877**, 4115-4124. https://doi.org/10.1016/j.jchromb.2009.11.004

[9] Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M. and Sadh, R.K. (2016) Somaclonal Variations and Their Applications in Horticultural Crops Improvement. 3 *Biotech*, **6**, Article No. 54. https://doi.org/10.1007/s13205-016-0389-7

[10] Punja, Z.K., Rodriguez, G. and Chen, S. (2017) Assessing Genetic Diversity in *Cannabis Sativa* Using Molecular Approaches. In: Chandra, S., Lata, H. and ElSohly, M., Eds., *Cannabis Sativa* L. *Botany and Biotechnology*, Springer, Cham, 395-418. https://doi.org/10.1007/978-3-319-54564-6_19

[11] Hale, J. (2020) Pollen from Hermaphroditic Flowers Can Pollinize, Study Shows. https://mjbizdaily.com/hermaphrodites-in-cultivated-cannabis-pollination-possible/

[12] Yang, R., Berthold, E.C., McCurdy, C.R., da Silva Benevenute, S., Brym, Z.T. and Freeman, J.H. (2020) Development of Cannabinoids in Flowers of Industrial Hemp (*Cannabis sativa* L.): A Pilot Study. *Journal of Agricultural and Food Chemistry*, **68**, 6058-6064. https://pubs.acs.org/doi/10.1021/acs.jafc.0c01211 https://doi.org/10.1021/acs.jafc.0c01211