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Ex Vitro Rooting of *Cannabis sativa* Microcuttings and Their Performance Compared to Retip and Stem Cuttings

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Abstract. **There is demand for micropropagated *Cannabis sativa* liner plants, because they are uniform, vigorous, and pathogen free; however, availability is limited because of challenges with in vitro culture decline and ex vitro rooting. Ex vitro rooting success of microcuttings was evaluated for 'Abacus' and 'Wife' when cultures were 6, 9, 12, 15, and 18 weeks old from initiation. Microcuttings of 'Wife' harvested from 6, 9, and 12-week-old cultures rooted at or above 80%, but rooting declined to 50% and 30% for 15- and 18-week-old cultures, respectively. Rooting for 'Abacus' remained relatively constant between 47% and 70% for microcuttings harvested from 6- to 18-week-old cultures. 'Wife' plants grown from microcuttings, stem cuttings, and retip cuttings (cuttings taken from new shoots on recently micropropagated plants) had equivalent total shoot length, number of shoots, and flower dry weight, whereas micropropagated 'Abacus' plants had less shoot length and flower dry weight than plants from stem cuttings. However, when micropropagated 'Abacus' plants were provided an extra week of vegetative growth to reach an initial size equivalent to stem and retip plants, all plants performed the same. Propagation method did not change cannabinoid content for both 'Abacus' and 'Wife'. Retip cuttings of 'Abacus' and 'Wife' rooted at 76% to 81% without rooting hormone, which is comparable to rates reported for stem cuttings of *C. sativa* treated with rooting hormone. Propagators should consider retipping to expand their liner production, because retips root well and possess the same desirable attributes as micropropagated plants.**

Many commercial *C. sativa* growers rely on mother stock plants and stem cuttings for propagation of liner plants (Adhikary et al. 2021; Monthony et al. 2021). There are limitations with this propagation method, because mother plants accumulate pathogens and lose vigor over time due to the serial removal of shoots for cuttings (Page et al. 2020). As a result, growers must replace mother plants every 3 to 6 months to maintain rooting success of cuttings (Lubell-Brand et al. 2021).

There is interest in micropropagation of *C. sativa* for the production of uniform, vigorous, and pathogen-free clones (Adhikary et al. 2021; Chandra et al. 2020). However, hyperhydricity, culture decline, and poor ex vitro rooting have limited the availability of micropropagated liner plants (De Klerk 2002; Monthony et al. 2021). Lubell-Brand et al. (2021) published a method that controlled hyperhydricity and extended culture life up to 15 weeks in vitro using a novel medium composition for shoot multiplication. The Lubell-Brand et al. (2021) method included the process of retipping, which is the repeated harvesting of new shoots from recently micropropagated plants that are then stuck as cuttings called retips (Keith and Brand 1995). Retipping can enhance output from the micropropagation process while still producing clonal, uniform liner plants. The objective of this work was to investigate how the age of in vitro *C. sativa* cultures micropropagated by the method of Lubell-Brand et al. (2021) affects ex vitro rooting success. An additional objective was to determine whether micropropagated plants and plants from retipping perform similarly to plants from stem cuttings in a greenhouse growing environment.

In some parts of the United States and Canada, the use of plant growth regulators (PGRs) for propagation is prohibited for *C. sativa* (Caplan et al. 2018; Feldman 2015; MDAR 2018), even though the PGR auxin has been found to enhance rooting success for some *C. sativa* cultivars (Campbell et al. 2021; Caplan et al. 2018). The use of Clonex® Rooting Gel (Hydrodynamics International, Lansing, MI) containing the a.i. indole-3-butyric acid (IBA), an auxin, at 3000 ppm, is US Environmental Protection Agency (EPA) registered and approved for use on all plants including food and medicinal crops (EPA 2016; HDI 2022). Lubell-Brand et al. (2021) demonstrated that retips of *C. sativa* routinely root at greater than 90% using IBA at 1000 ppm. A third objective was to evaluate rooting ability of retips treated with and without IBA to demonstrate the versatility of this alternative cloning method.

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Materials and Methods

Ex vitro rooting timing study. 'Wife' and 'Abacus' were initiated in tissue culture from stock plants maintained in a greenhouse following the methods outlined in Lubell-Brand et al. (2021). The same micropropagation boxes and growth chamber environment used by Lubell-Brand et al. (2021) were used here. For each cultivar, a total of 42 boxes were initiated and each box contained four shoots on initiation medium consisting of Murashige and Skoog (MS) with vitamins medium plus 3% sucrose, 0.5 $mg \cdot L^{-1}$ metatopolin (MT) and 1% (w/v) agar at pH 5.7. After 3 weeks, shoots were subcultured to fresh initiation medium and grown for another 3 weeks. After the 6 weeks in initiation medium, five boxes of each cultivar, selected at random, were subcultured to prerooting medium consisting of MS with vitamin medium plus 3% sucrose, 1 $mg \cdot L^{-1}$ IBA, and 0.8% (w/v) agar at pH 5.7. The remaining boxes were subcultured to shoot multiplication consisting of MS with vitamin medium plus 3% sucrose, 0.5 $mg \cdot L^{-1}$ MT, 0.1 $mg \cdot L^{-1}$ gibberellic acid, and 0.8% (w/v) agar at pH 5.7. From then on, every 3 weeks up to 18 weeks, five boxes of each cultivar were subcultured to prerooting medium and the remaining boxes were subcultured to shoot multiplication medium. After 2 weeks in prerooting medium, microcuttings were transferred ex vitro for rooting to 1-inch rockwool cubes that were set in sections of 96 plug trays consisting of four plug cells that fit in plastic salad trays. The experimental unit (EU) was a tray with four microcuttings. Trays were arranged in a randomized complete block design (RCBD) with five replications. As new trays were prepared every 3 weeks, from weeks 6 to 18 of this study, trays were re-randomized appropriately. Trays were maintained under a 24-h photoperiod provided by LED lamps at an intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 4 weeks in rockwool, percent rooting per tray was recorded.

Greenhouse performance of microcutting, retip, and stem cutting plants study. This study was conducted twice. The first experiment compared microcuttings and stem cuttings and the second experiment compared microcuttings, stem cuttings, and retip cuttings. For the first experiment, microcuttings from the ex vitro rooting timing study were used. Rooted microcuttings in trays were acclimated to greenhouse conditions as described by Lubell-Brand et al. (2021). Stem cuttings from mother stock plants were taken the same day microcuttings were transferred ex vitro to rockwool. Stem cuttings were dipped in talc-based IBA at 2000 ppm (Hormodin #2, OHP, Mainland, PA, USA), stuck into 1-inch rockwool cubes set in 96 plug trays, covered with clear plastic 6-inch propagation domes and allowed to root for 4 weeks under a 24-h photoperiod as described. For acclimation to greenhouse conditions, propagation domes were gradually vented to decrease relative humidity around rooted cuttings, as described by Lubell-Brand et al. (2021). Rooted stem cuttings and microcuttings were potted in 2-gal

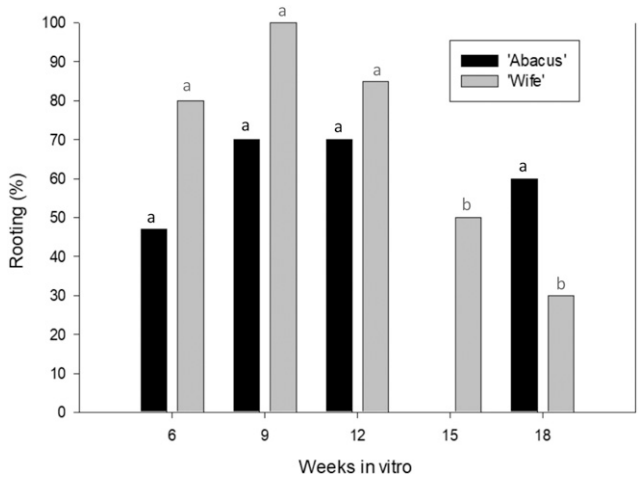

Fig. 1. Ex vitro rooting percent for microcuttings of *Cannabis sativa* 'Abacus' and 'Wife' taken when cultures were 6, 9, 12, 15, and 18 weeks old from initiation. Microcuttings of 'Abacus' at 15 weeks became diseased with botrytis and died, therefore rooting data could not be collected. Mean separation within cultivar, indicated by different letters, using Fisher's least significant difference test at $P \leq 0.05$ (n = 5).

containers with peat based medium (metro-mix 830; Sungro, Agawam, MA, USA) and top dressed with 32 g of 15N–3.9P–10K controlled-release fertilizer (Osmocote Plus 5- to 6-month formulation; Everris NA, Dublin, OH, USA). At time of potting, rooted stem cuttings had an initial shoot length of 12 cm, and rooted microcuttings 5 cm. On day 15 after potting, all plants had the distal 2.5 cm of the primary shoot pruned off to relieve apical dominance and promote axillary branching. The EU was a single potted plant and plants were arranged in an RCBD with six replications. Plants were allowed to grow vegetatively under long-day (18-h) conditions provided by supplemental lighting with 1000-W high-pressure sodium (HPS) lamps (Phantom HPS 100W; Hydrofarm, Petaluma, CA, USA) for 20 d. Flowering was induced by providing short-day (12-h) conditions for 40 d using black-out curtain. During long-day conditions, plants were fertigated with a soluble fertilizer (Peters 20N–8.7P–16.6K; Scotts, Marysville, OH, USA), providing 100 ppm nitrogen (N) at each watering. During short-day conditions, plants were fertigated with a higher P soluble fertilizer (Peters 15N–12.9P–12.5K; Scotts) providing 100 ppm N at each watering. The number of shoots, total shoot length, and inflorescence dry weight per plant were recorded. To determine inflorescence dry weight, plants were dried at room temperature for 2 weeks, then inflorescences were separated from leaves and stems and weighed. Cannabinoid content of dried flower was analyzed by high-performance liquid chromatography at The University of Connecticut Center for Environmental Sciences and Engineering (Storrs, CT, USA).

For the second experiment, propagation of microcuttings and stem cuttings were as described. Retip cuttings (5 to 7 cm) were taken from 17-day-old micropropagated plants that had been pruned once at 10 d after potting and handled as described by Lubell-Brand et al. (2021). Rooted cuttings were potted as in Expt. 1. The EU was a single potted plant and units were arranged as an RCBD with six replications. Microcuttings were observed to be slower to initiate shoot growth than stem cuttings in Expt. 1, so in Expt. 2, microcuttings were transferred to rockwool and potted 7 d earlier than retips and stem cuttings to provide them with more time under long-day conditions for vegetative growth. Plants from retips and stem cuttings received 15 d of long days and plants from microcuttings received 22 d of long days. All plants received 35 d of short-day conditions. At 14 d before beginning short days, plants had their primary shoot pruned so that 4 to 5 nodes remained and plants were 7 to 9 cm tall. Data collection was as described for Expt. 1. In addition, total dry weight of stems, leaves, and inflorescences was recorded, and stem caliper, measured between the third and fourth node from the stem base, was recorded.

Retip cutting auxin study. Micropropagated plants of 'Abacus' and 'Wife' to be used to supply retips for this experiment were pruned and allowed to grow out twice before retips were taken. Retips were taken on days 24 (time replication 1) and 31 (time replication 2) after plants were potted and moved to the greenhouse. Retip cuttings were treated with 0 or 1000 ppm IBA (Hormodin #1; OHP) and rooted as described. The EU was four retip cuttings. An RCBD was used with 12 replications per time replication. Four weeks after transfer to rockwool, percent rooting per EU was recorded.

Data analysis. Data were subjected to analysis of variance (PROC GLIMMIX) and mean separation with Fisher's least significant difference test ($P \leq 0.05$) using SAS (version 9.4; SAS Institute, Cary, NC, USA). Interaction effects were reported, because they were significant for some dependent variables.

Results and Discussion

Microcuttings of 'Wife' harvested from 6-, 9-, and 12-week-old cultures rooted at or above 80% (Fig. 1). Rooting declined to 50% for microcuttings harvested from 15-week-old

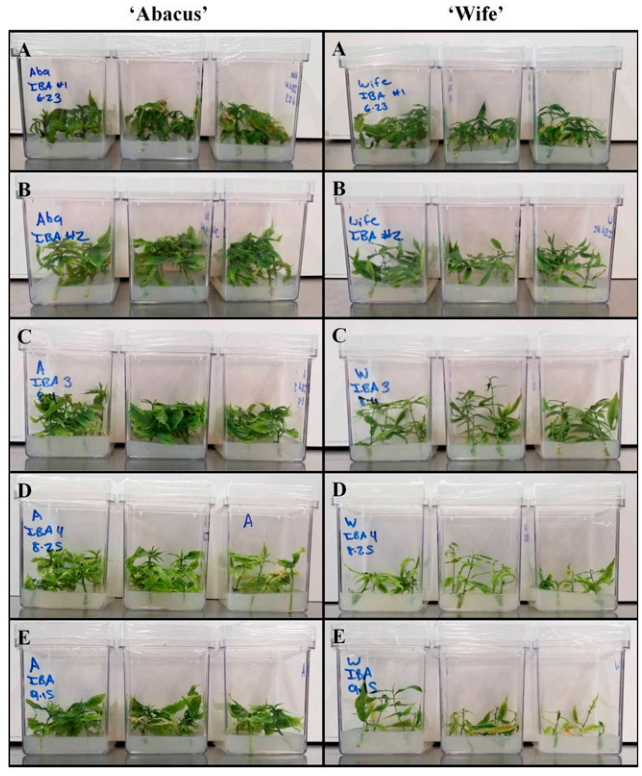

Fig. 2. Microcuttings of *Cannabis sativa* 'Abacus' and 'Wife' after 2 weeks in prerooting medium and just before transfer ex vitro to rockwool for cultures aged (**A**) 6 weeks, (**B**) 9 weeks, (**C**) 12 weeks, (**D**) 15 weeks, and (**E**) 18 weeks.

cultures and 30% for 18-week-old cultures. Rooting for 'Abacus' remained relatively constant between 47% and 70% for microcuttings harvested from 6- to 18-week-old cultures. Microcuttings of 'Abacus' at 15 weeks became diseased with botrytis and died, therefore rooting data could not be collected. Marginal yellowing of leaves, indicating a possible nutrient imbalance, was noticeable on microcuttings of 'Wife' and 'Abacus' taken from 15- and 18-week-old cultures (Fig. 2). In their review of *C. sativa* tissue culture, Monthony et al. (2021) identified seven reports that discuss in vitro rooting response, which ranged from 44% to 95%, and no reports that describe ex vitro rooting success rates. Ex vitro rooting is preferred over in vitro rooting, because ex vitro rooted plants have better-developed root systems and greater chances of survival (Hartmann et al. 2002).

In greenhouse performance Expt. 1, micropropagated 'Wife' plants produced the same shoot length, number of shoots, and flower dry weight as plants propagated from stem cuttings (Table 1). Micropropagated 'Abacus' had fewer shoots and less flower dry weight than plants from stem cuttings. Despite their smaller initial size, micropropagated 'Abacus' plants grew vigorously over the duration of the experiment, with an increase in shoot length of 6800% from the beginning of the study. 'Abacus' plants from stem cuttings increased in shoot length by 5000% from the beginning of the study. When micropropagated plants were provided an additional week of vegetative growth in Expt. 2, micropropagated 'Abacus' plants were equivalent to plants from stem and retip cuttings for all measured parameters, except stem caliper (Table 2; Fig. 3). Micropropagated 'Wife' plants in Expt. 2 exceeded plants from stem and retip cuttings for total dry weight and flower dry weight. Propagation method did not change cannabinoid content for both 'Abacus' and 'Wife' (Tables 1 and 2). Other studies on *C. sativa* have reported no differences in cannabinoid content between tissue-cultured plants, plants propagated from stem cuttings, and/or mother plants (Chandra et al. 2009; Lata et al. 2016).

Several studies have demonstrated for crops including blueberry (*Vaccinium angustifolium*), cherry (*Prunus avium*), strawberry (*Fragaria ×ananassa*), mulberry (*Morus indica*), eucalyptus (*Eucalyptus grandis ×urophylla*), lingonberry (*Vaccinium vitis-idaea*), ginger (*Zingibar officinale*), and others that micropropagated plants perform similarly to, or better than, plants from stem cuttings for yield, survival, and biochemical content (Capocasa et al. 2019; Chitra et al. 2016; Gustavsson and Stanys 2000; Hammatt 1999; Jamieson and Nickerson 2003; Ma and Gang 2006; Yang et al. 1995; Zaman et al. 1997). Capocasa et al. (2019), working with the well-known strawberry cultivar 'Alba', demonstrated that micropropagated plants were equivalent to conventional in vivo mother plants for frigo plant nursery production. Although micropropagated plants were smaller than in vivo plants for some vegetative traits, the researchers state that if micropropagated plants were provided more time to develop before planting in the nursery, then their use as mother plants would lead to increased production of new runners.

The results provided here, and work by other researchers, demonstrate that *C. sativa* cultivars perform differently in vitro (Monthony et al. 2021; Page et al. 2020). Some cultivars, like 'Wife', will reach a maximum rooting rate not long after initiation to in vitro culture, and then steadily decline to inadequate rooting levels. Other cultivars, like 'Abacus', will root at an adequate level over an extended period of time in vitro. For cultivars like 'Abacus' that are slow to begin rapid growth following

Table 1. Total shoot length, number of shoots, flower dry weight, and percent cannabidiol (CBD) and tetrahydrocannabinol (THC) for plants of *Cannabis sativa* 'Abacus' and 'Wife' from microcuttings or stem cuttings. Plants were grown in a greenhouse under long days (18-h) for 20 d and then short days (12-h) for 40 d.

	Total shoot length (cm)	No. shoots	Flower dry wt (g)	CBD (%)	THC (%)
n	6	6	3	3	3
'Abacus'					
Microcuttings	345.7 a[i]	10.5 b	54.9 b	13.9 a	0.6 a
Stem cuttings	609.6 a	18.4 a	82.2 a	13.5 a	0.6 a
'Wife'					
Microcuttings	1058.0 a	26.2 a	66.5 a	11.0 a	0.5 a
Stem cuttings	1082.0 a	23.6 a	64.7 a	10.4 a	0.5 a

[i] Mean separation within cultivar within column, indicated by different letters, by Fisher's least significant difference test at $P \leq 0.05$.

Table 2. Stem caliper, number of shoots, total shoot length, total dry weight, flower dry weight, and percent cannabidiol (CBD) and tetrahydrocannabinol (THC) for plants of *Cannabis sativa* 'Abacus' and 'Wife' from microcuttings, retip cuttings, or stem cuttings. Plants from retip cuttings and stem cuttings were grown in a greenhouse under long days (18 h) for 15 d and plants from microcuttings for 22 d. All plants received 35 d of short days (12 h).

	Stem caliper (mm)	No. shoots	Total shoot length (cm)	Total dry wt (g)	Flower dry wt (g)	CBD (%)	THC (%)
n	6	6	6	6	6	5	5
'Abacus'							
Microcuttings	3.3 b[i]	10.2 a	282.2 a	68.9 a	21.9 a	10.4 a	0.4 a
Retip cuttings	4.3 a	7.2 a	214.0 a	77.3 a	20.8 a	9.6 a	0.4 a
Stem cuttings	4.3 a	8.3 a	258.5 a	92.5 a	25.4 a	9.1 a	0.4 a
'Wife'							
Microcuttings	4.9 a	18.5 a	789.2 a	150.9 a	32.5 a	7.5 a	0.3 a
Retip cuttings	4.6 a	16.2 a	617.0 a	115.1 b	23.4 b	7.2 a	0.3 a
Stem cuttings	4.6 a	18.5 a	690.2 a	120.8 b	24.9 b	6.2 a	0.3 a

[i] Mean separation within cultivar within column, indicated by different letters, by Fisher's least significant difference test at $P \leq 0.05$.

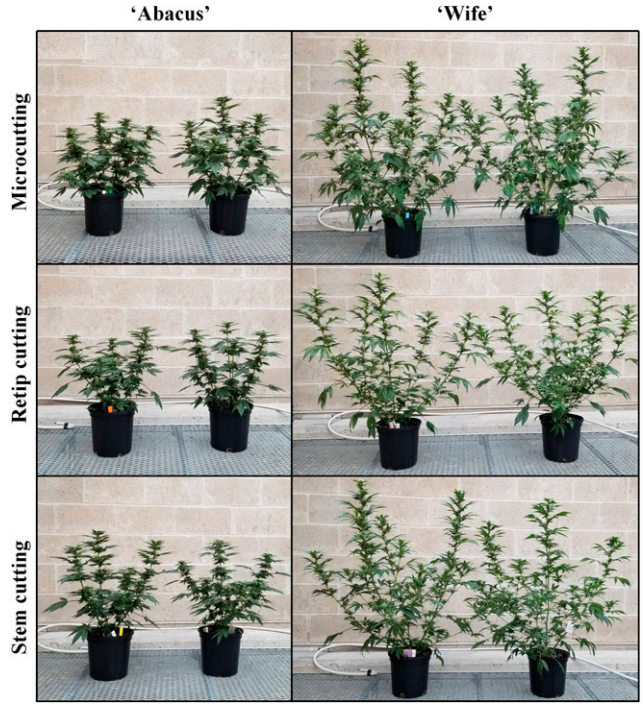

Fig. 3. Flowering plants of *Cannabis sativa* 'Abacus' and 'Wife' from microcuttings, retip cuttings, and stem cuttings at 35 d of growth under short-day (12-h) conditions.

Table 3. Percent rooting for retip cuttings of *Cannabis sativa* 'Abacus' and 'Wife' treated with 0 or 1000 ppm indole-3-butyric acid (IBA).

IBA (ppm)	Time replication 1 Rooting (%)	Time replication 2 Rooting (%)
0	76 a[i]	81 a
1000	85 a	88 a

[i] Mean separation within column indicated by different letters, by Fisher's least significant difference test at $P \leq 0.05$ (n = 24).

acclimation to greenhouse conditions, additional time under long-day conditions will benefit performance.

There are a limited number of published reports about cutting propagation for *C. sativa*. A recent study by Campbell et al. (2021) evaluated eight commercial cannabidiol cultivars and found that auxin applications significantly improved rooting success compared with no hormone application for all cultivars. Conversely, McLeod et al. (2022) showed that hormone had no effect on rooting success of stem cuttings of *C. sativa* 'I3'. We demonstrated that retip cuttings of 'Abacus' and 'Wife', treated with and without exogenous auxin at 1000 ppm IBA, root similarly at 76% to 88% (Table 3). A comparable success rate (84%) was reported by Caplan et al. (2018) for stem cuttings of *C. sativa* 'WP:Med (Wappa)' treated with 2000 ppm IBA. Our results demonstrate that retip cuttings can be as successful as stem cuttings for producing liner plants of *C. sativa*. Propagators of *C. sativa* should consider retipping to expand their production of liner plants, which possess all of the positive characteristics of micropropagated plants.

References

Adhikary, D., M. Kulkarni, A. El-Mezawy, S. Mobini, M. Elhiti, R. Gjuric, A. Ray, P. Polowick, J.J. Slaski, M.P. Jones, and P. Bhowmik. 2021. Medical cannabis and industrial hemp tissue culture: Present status and future potential. Front Plant Sci. 12:627240, https://doi.org/10.3389/fpls.2021.627240.

Campbell, S.M., S.L. Anderson, Z.T. Brym, and B.J. Pearson. 2021. Evaluation of substrate composition and exogenous hormone application on vegetative propagule rooting success of essential oil hemp. PLoS One 16(7):e0249160, https://doi.org/10.1371/journal.pone.0249160.

Caplan, D., J. Stemeroff, M. Dixon, and Y. Zheng. 2018. Vegetative propagation of cannabis by stem cuttings: Effects of leaf number, cutting position, rooting hormone, and leaf tip removal. Can. J. Plant Sci. 98:1126–1132, https://doi.org/10.1139/cjps-2018-0038.

Capocasa, F., F. Balducci, M. Marcellini, D. Bernardini, O. Navacchi, and B. Mezzetti. 2019. Comparing nursery behavior, field plant yield, and fruit quality of in vitro and in vivo propagated strawberry mother plants. Plant Cell Tissue Organ Cult. 136:65–74, https://doi.org/10.1007/s11240-018-1492-8.

Chandra, S., H. Lata, Z. Mehmedic, I.A. Khan, and M.A. Elsohly. 2009. Assessment of cannabinoids in micropropagated plants of *Cannabis sativa* and their comparison with conventionally propagated mother plant during developmental stages of growth. Planta Med. 76(7):743–750, https://doi.org/10.1055/s-0029-1240628.

Chandra, S., H. Lata, and M.A. Elsohly. 2020. Propagation of *Cannabis* for clinical research: An approach towards a modern herbal medicinal products development. Front Plant Sci. 11:958, https://doi.org/10.3389/fpls.2020.00958.

Chitra, D.S.V., B. Chinthapalli, and G. Padmaja. 2016. A comparative study on field performance of micropropagated plants and stem cutting derived plants of S-36 cultivar of mulberry (*Morus indica* L.). Res. Biotechnol. 7(1):21–27, https://doi.org/10.19071/rib.2016.v7.2930.

De Klerk, G.J. 2002. Rooting of microcuttings: Theory and practice. In Vitro Cell. Dev. Biol. 38:415–422, https://doi.org/10.1079/IVP2002335.

Feldman, J. 2015. Pesticide use in marijuana production: Safety issues and sustainable options. Pesticides and You 34(4):14–23.

Gustavsson, B.A. and V. Stanys. 2000. Field performance of 'Sanna' lingonberry derived by micropropagation vs. stem cuttings. HortScience 35(4):742–744, https://doi.org/10.21273/HORTSCI.35.4.742.

Hammatt, M. 1999. Delayed flowering and reduced branching in micropropagated mature wild cherry (*Prunus avium* L.) compared with rooted cuttings and seedlings. Plant Cell Rep. 18:478–484, https://doi.org/10.1007/s002990050607.

Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve. 2002. Hartmann & Kester's plant propagation: Principles and practices (7th ed). Prentice Hall, Upper Saddle River, NJ, USA.

Hydrodynaics International (HDI). 2022. Clonex Rooting Gel has been used to root billions of clones across the planet. https://www.hydrodynamicsintl.com/learn-facts-clonex-rooting-gel/. [accessed 19 Aug 2022].

Jamieson, A.R. and N.L. Nickerson. 2003. Field performance of the lowbush blueberry propagated by seed, stem cuttings and micropropagation. Acta Hort. 626:423–428, https://doi.org/10.17660/actahortic.2003.626.58.

Keith, V.M. and M.H. Brand. 1995. Influence of culture age, cytokinin level, and retipping on growth and incidence of brooming in micropropagated rhododendrons. J. Environ. Hortic. 13:72–77, https://doi.org/10.24266/0738-2898-13.2.72.

Lata, H., S. Chandra, N. Techen, I.A. Khan, and M.A. Elsohly. 2016. In vitro mass propagation of *Cannabis sativa* L.: A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants. J. Appl. Res. Med. Aromat. Plants 3:18–26, https://doi.org/10.1016/j.jarmap.2015.12.001.

Lubell-Brand, J.D., L.E. Kurtz, and M.H. Brand. 2021. An in vitro-ex vitro micropropagation system for hemp. HortTechnology 31(2):199–207, https://doi.org/10.21273/HORTTECH04779-20.

Ma, X. and D.R. Gang. 2006. Metabolic profiling of in vitro micropropagated and conventionally greenhouse grown ginger (*Zingiber officinale*). Phytochemistry 67(20):2239–2255, https://doi.org/10.1016/j.phytochem.2006.07.012.

Massachusetts Department of Agricultural Resources (MDAR). 2018. Pesticide use on marijuana and hemp. https://www.mass.gov/doc/pesticide-use-on-marijuana-and-hemp/download. [accessed 20 Jul 2022].

McLeod, A., K. Vining, T. Hoskins, and R. Contreras. 2022. Impact of indole-3-butyric acid concentration and formulation and propagation environment on rooting success of "I3" hemp by stem cuttings. HortTechnology 32(3):321–324, https://doi.org/10.21273/HORTTECH05016-21.

Monthony, A.S., S.R. Page, M. Hesami, and A.M.P. Jones. 2021. The past, present and future of *Cananbis sativa* tissue culture. Plants 10:1–28, https://doi.org/10.3390/ plants10010185.

Page, S.R., A.S. Monthony, and A.M.P. Jones. 2020. DKW basal salts improve micropropagation and callogenesis compared to MS basal salts in multiple commercial cultivars of *Cannabis sativa*. Botany 99:269–279, https://doi.org/10.1139/cjb-2020-0179.

U.S. Environmental Protection Agency (EPA). 2016. Labels for CLONEX ROOTING GEL (79664-1). https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:102:NO:P102_REG_NUM:79664-1. [accessed 19 Aug 2022].

Yang, J.C., J.D. Chung, and Z.Z. Chen. 1995. Vegetative propagation of adult *Eucalyptus grandis x urophylla* and comparison of growth between micropropagated plantlets and rooted cuttings. Plant Cell Rep. 15:170–173, https://doi.org/10.1007/BF00193713.

Zaman, A., R. Islam, and O.I. Joarder. 1997. Field performance and biochemical evaluation of micropropagated mulberry plants. Plant Cell Tissue Organ Cult. 51:61–64, https://doi.org/10.19071/rib.2016.v7.2930.