Impact of Indole-3-butyric Acid Concentration and Formulation and Propagation Environment on Rooting Success of ‘I3’ Hemp by Stem Cuttings

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Summary. As the industrial hemp (*Cannabis sativa* L.) market grows, there is a need for methods to clonally propagate parental breeding stock and new cultivars. Information is lacking on vegetative cutting propagation of hemp. We evaluated how propagation environment (intermittent mist vs. subirrigation under a humidity dome), indole-3-butyric acid (IBA) formulation (talc rooting powder vs. IBA in solution), and IBA concentration (0, 3000, or 8000 ppm) affected stem cuttings from ‘I3’, a cannabinoid-free cultivar of industrial hemp. Under mist or domes, rooting quality and percent declined at 8000 ppm IBA. Root and shoot quality and rooting percentage also were reduced in 3000 ppm IBA in solution treatment compared with talc. Our data show that for the cultivar tested, cuttings rooted at the highest percentage and produced the highest-quality roots and shoots with either no hormone or 3000 ppm talc powder. These treatments did equally well under humidity domes or intermittent mist.

The non-recreational hemp (*Cannabis sativa* L.) industry, particularly the cannabidiol sector, is expanding rapidly. Commercial growers and researchers are shifting to sterile triploids to avoid pollen contamination from male hemp plants grown nearby. As such, producers need reliable and efficient methods to clonally propagate industrial hemp on a large scale. Hemp is propagated either by seed (Potter, 2009), by stem cuttings (Caplan, 2018), or in vitro (Lata et al., 2017). Stem cuttings are a common method for propagation of hemp, but many factors affect the success and quality of the cuttings.

During vegetative propagation, transpiration is minimized by using methods like intermittent mist or humidity domes to maintain a high relative humidity around cuttings until they produce roots. If the relative humidity is too low, transpiration will be increased, which can cause cuttings to wilt faster and die (Owen, 2018). Humidity domes have previously been used in other experiments involving the propagation of woody plant species, such as aspen (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.) (Wolken et al., 2010). Intermittent mist systems are the most common method of increasing humidity during cutting propagation. These systems apply short bursts of water in small droplets to the plants every few minutes throughout the duration of the light cycle. The water bursts help to keep humidity high and to minimize the rate of transpiration. Intermittent mist further reduces vapor pressure deficit by cooling the surface of the leaf via evaporation. Humidity domes (Campbell et al., 2019; Parsons et al., 2019) and intermittent mist systems (Clarke, 1981) have both been used in hemp propagation.

Indole-3-butyric acid (IBA) is often used for rooting in commercial operations (De Klerk et al., 1999) and is available in various formulations, concentrations, and application methods. IBA can be delivered to cuttings in talc or dissolved in alcohol to be used as a quick dip, whereas the potassium salt of IBA can be dissolved in water alone. Caplan (2018) found that a 0.2% (2000 ppm) IBA gel applied to hemp cuttings doubled the rooting percentage when compared with a 2000-ppm solution of willow (*Salix* sp.) extract. Although growers may opt to use talc or liquid formulations, it would be useful for growers to see how a single hemp breeding line reacts to each.

The purpose of our study was to evaluate the impact of environment (dome vs. intermittent mist), IBA formulation [talc vs. IBA/naphthalene acetic acid (NAA) quick dip], and IBA concentration on rooting percent and the root and shoot quality of stem cuttings from ‘I3’ hemp.

Materials and methods

Plant material. Stock plants were maintained in a greenhouse under a 24-h photoperiod with a mean canopy light intensity of 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using 400-W high-pressure sodium lamps (Sun System, Vancouver, WA). Stock plants were potted at the beginning of Oct. 2020 as rooted cuttings in 5-gal containers. The containers were filled with a soilless potting mix (Metro-Mix; Sun Gro Horticulture, Agawam, MA) and perlite (Supreme Perlite Co., Portland, OR) (2:1 by volume)and incorporated with 67.5 g of 18N–2.6P–9.1K controlled-release fertilizer (Harrell’s, Lakeland, FL) per 2 ft^3 of soilless potting mix (Metro-Mix). Plants were fertilized weekly with water-soluble 20N–8.7P–16.6K general-purpose fertilizer (Jack’s Professional; JR Peters, Allentown, PA) at 100-ppm concentration measured by a water-powered, non-electric chemical

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.0283	ft^3	m^3	35.3147
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

injector (Dosatron; Dosatron International, Clearwater, FL). The stock plants were 5 months old when cuttings were collected at the end of Feb. 2021. A mixture of terminal and subterminal cuttings was collected at 1400 HR. All cuttings were ≈4–5 inches in length, and each cutting had two or three fully expanded leaves. Terminal and subterminal cuttings were randomly assigned to each treatment combination. Cuttings were rooted in 10- by 20-inch plastic trays (Hydro Crunch, Walnut, CA) with drainage that contained soilless media (Sunshine Mix, Sun Gro Horticulture) and perlite (Supreme Perlite Co.) (2:1 by volume). Each drainage tray was set inside of a solid bottom 10-inch by 20-inch plastic tray (Hydro Crunch) that held water for subirrigation. The water in the bottom tray moved up through the medium by capillary action. Twenty-five cuttings were placed in each tray.

Experimental design and environment. The experimental design was a randomized complete block design with a split plot arrangement of the environmental treatments. Each individual tray with 25 cuttings was counted as an experimental unit. A plastic-tented mist bench was used for the experiment. The bench was divided in half between the two environments (mist vs. no mist), and the remaining treatments were randomized within those two sub-plots. One half of the tented mist bench was equipped with mist emitters (CoolNet Pro Fogger; Netafim USA, Fresno, CA) suspended ≈30 inches above the bench surface, and the other half of the bench did not have any mist emitters; this half was used for the humidity domes. There were two IBA formulations and three different IBA concentrations applied to cuttings in both propagation methods. There were three replications for each of the 12 treatment combinations. Thirty-six different experimental units (trays) were evaluated, and a total of 900 cuttings were used. The IBA formulations were talc rooting powder (Hormex; Brooker Chemical Corp., Chatsworth, CA) and a mixture of 10,000 ppm IBA and 5000 ppm NAA in solution (Wood's Rooting Compound; Earth Science Products Corp., Wilsonville, OR). Both IBA formulations were applied at 0, 3000, and 8000 ppm.

Cuttings were collected, treated, and placed in the tented mist bench on 25 Feb. 2021. The tented mist bench was inside of a climate-controlled glass greenhouse with day/night set temperatures of 26/15 °C, with no supplemental lighting, and bottom heat at 22 °C. The trays were randomized within each section (mist vs. no mist). Trays in each group were watered at the time of propagation. From that point on, the humidity dome group was sub-irrigated with tap water as needed. The intermittent mist group was misted for 12 s once every 45 min from 0700 to 2000 HR. The humidity domes each had two vents on top. For days 1–5 after inserting cuttings into the growing medium, both vents were kept closed. On days 6–10, both vents were opened 25%. On days 11–15, both vents were opened 50%. On day 16, both vents were fully opened and remained open until the cuttings were harvested on day 28 (24 Mar. 2021).

Assessing root and shoot quality. Twenty-eight days after initiation, all cuttings were harvested, their root and shoot quality were assessed, and a rooting percentage was calculated. Root and shoot quality were assessed by rating the shoot and root system for each cutting on a scale

Fig. 1. Quality ratings used to evaluate (A) shoot quality and (B) root quality 4 weeks after treatment of 'I3' hemp cuttings. Quality was ranked on a 0–4 relative scale, with 4 representing the highest quality rating. A rating of 0 for shoots means that the shoot remained the same size as the initial cutting and did not produce any new leaves. A rating of 0 for roots means the cutting produced no roots.

Table 1. Mean rooting percentage of 'I3' hemp stem cuttings following 12 treatment combinations.

Treatment no.	Environment[z]	IBA formulation[y]	IBA concn (ppm)[x]	Mean rooting (%)
1	Mist	IBA in solution	0	88 abc[w]
2	Mist	IBA in solution	3000	72 c
3	Mist	IBA in solution	8000	76 bc
4	Mist	Talc powder	0	85 abc
5	Mist	Talc powder	3000	97 a
6	Mist	Talc powder	8000	83 abc
7	Dome	IBA in solution	0	89 abc
8	Dome	IBA in solution	3000	91 ab
9	Dome	IBA in solution	8000	87 abc
10	Dome	Talc powder	0	89 a
11	Dome	Talc powder	3000	89 a
12	Dome	Talc powder	8000	76 bc

[z]Environments tested were "Mist," which refers to intermittent mist, and "Dome," which refers to removable humidity domes.
[y]The two indole-3-butyric acid (IBA) formulations were "IBA in solution," which was an IBA/naphthalene acetic acid quick dip mixture, and "talc powder," which was commercial rooting powder (Hormex; Brooker Chemical Corp., Chatsworth, CA).
[x]1 ppm = 1 $mg \cdot L^{-1}$.
[w]Treatments that share letters within the response variable (mean rooting) were not significantly different based on Fisher's least significant difference (α = 0.05).

of 0–4 (Fig. 1), with 4 being the best quality. A rating of 0 for shoots means that the shoot remained the same size as the initial cutting and did not produce any new leaves. A rating of 0 for roots means the cutting produced no roots. The mean root quality score and shoot quality score were calculated from the group of 25 cuttings within each experimental unit and subjected to analysis of variance (ANOVA) using RStudio (ver. 4.0.2; Allaire Corp., Newton, MA). ANOVA was used to compare results among the different propagation environments (humidity dome vs. intermittent mist), IBA formulations (talc powder vs. IBA in solution), and IBA concentrations. To independently assess the main effect treatments (different combinations of propagation environment, IBA formulation, and IBA concentration), Tukey's honestly significant difference and Fisher's least significant difference tests were performed.

Results and discussion

Rooting success was high in both propagation environments, and there was no significant difference between them ($P = 0.28$); 88% of the cuttings propagated under the humidity domes rooted, and 84% of the cuttings under intermittent mist rooted (Table 1). However, there was a difference among all treatments ($P = 0.094$) if α was raised to 0.1. The propagation environment had no significant effect on root quality (Table 2). However, it had a significant, but modest, effect on shoot quality (Table 2). The average shoot quality rating for cuttings propagated under humidity domes was 1.85, and the average shoot quality rating for cuttings under intermittent mist was 1.62 [$P < 0.01$ (Fig. 2)]. Cuttings from the intermittent mist group were generally healthy, but mild amounts of chlorosis were observed on some leaves. Cuttings under the humidity domes did not show any signs of chlorosis. Other research has demonstrated that leaf chlorosis can occur under misting (Zhang and Graves, 1995).

There was no significant difference in rooting success ($P = 0.16$) between talc (88%) and IBA in solution (83%). These findings generally concur with those of Campbell et al. (2021), who evaluated the effects of five different hormone treatments that included three different IBA formulations used in hemp propagation [Hormodin (OHP, Bluffton, SC), Hormex, Dip'N Grow (Dip'N Grow, Clackamas, OR)] and reported that the different rooting hormone treatments used had no significant differences in terms of rooting success. IBA formulation had a significant effect on quality for both shoots and roots (Table 2). The average shoot quality rating for cuttings treated with talc rooting powder (2.0) was significantly different ($P < 0.001$) from the average for cuttings treated with IBA in solution (1.5). The average root quality rating for cuttings treated with talc rooting powder was 2.0, and for IBA in solution it was 1.7 ($P < 0.001$). If IBA is applied, talc powder is significantly more effective than the IBA solution we evaluated in terms of stimulating root and shoot development.

There was a significant difference in rooting percentage among the different

Table 2. Mean square error (MSE) of root and shoot quality of 'I3' hemp stem cuttings that were propagated in two different environments, with two different indole-3-butyric acid (IBA) formulations and with three different IBA concentrations.

Component of variance	df	MSE (roots)[z]	MSE (shoots)[z]
Environment	1	0.02	11.56*
IBA formulation	1	13.44**	50.89**
IBA concentration	2	20.97**	72.13**
Environment × IBA formulation	1	0.04	1.78
Environment × IBA concentration	2	0.03	0.54
IBA formulation × IBA concentration	2	8.51**	19.67**
Environment × IBA formulation × IBA concentration	2	3.08	1.03
Residuals	888	1.2	1.09

[z]MSE (in rows and columns) subjected to by analysis of variance.
*, **Significant at $P < 0.01$ or 0.001, respectively.

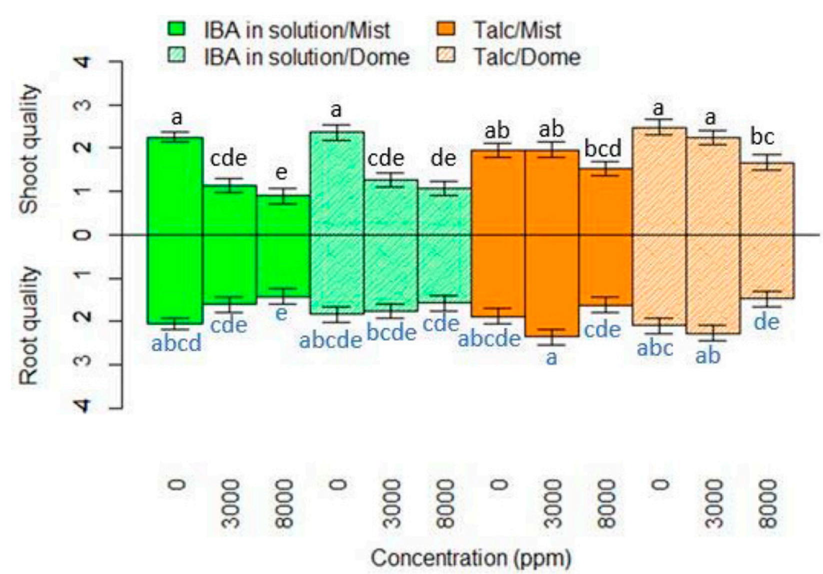

Fig. 2. A representation of the two-factor indole-3-butyric acid (IBA) formulation × IBA concentration interaction. Root and shoot quality are presented on a 0–4 relative scale for 'I3' hemp stem cuttings, with 4 being the best quality. A rating of 0 for shoots means that the shoot remained the same size as the initial cutting and did not produce any new leaves. A rating of 0 for roots means the cutting produced no roots. Ratings are separated by treatment combinations. The IBA concentration used in each treatment combination is stated below each treatment combination. "IBA in solution" represents the IBA/naphthalene acetic acid quick dip mixture. "Mist" refers to the intermittent mist propagation environment. "Dome" refers to the humidity dome propagation environment. "Talc" refers to talc based IBA powder. Shoot and root quality ratings are the two different response variables. Error bars represent standard error of the mean. Bars within each response variable (root or shoot quality) with the same letter are not different based on Tukey's honest significant difference test ($\alpha = 0.05$). Black letters refer to shoot quality; blue letters refer to root quality; 1 ppm = 1 mg·L^{-1}.

concentrations (P = 0.02). The 0- and 3000-ppm treatments both led to an average rooting percentage of 89%, whereas the average rooting percentage of the 8000-ppm treatment was reduced to 79%. This contrasts with Campbell et al. (2021), who reported a 15- to 18-fold increase in rooting with hormone compared with no hormone. IBA concentration had a significant effect ($P < 0.001$) on the quality scores of both roots and shoots (Table 2, Fig. 2). The average shoot quality scores for cuttings were 3.3 for the 0-ppm treatment, 2.7 for the 3000-ppm treatment, and 2.3 for the 8000-ppm treatment. The average root quality scores were 3.0 for the 0-ppm treatment, 3.0 for the 3000-ppm treatment, and 2.5 for the 8000-ppm treatment. At 8000 ppm, both compounds were less effective than at 3000 ppm or control. There were no differences in cutting response between the controls and the 3000-ppm talc treatment. Applying 8000 ppm had a negative impact on root and shoot quality (Fig. 2).

The IBA formulation × IBA concentration interaction was significant in both root and shoot quality (Table 2). The interaction was observed at 3000 ppm, where the average quality of cuttings treated with IBA in solution decreased significantly, whereas those treated with talc powder improved.

In conclusion, our research shows that growers can successfully root stem cuttings by using both humidity domes and intermittent mist and without using rooting hormone. We found that 3000 ppm talc powder was superior to IBA in solution at the concentrations we tested and found that 8000 ppm was detrimental to success, regardless of formulation.

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