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Vegetative Propagation of Perennial Cut Flower Flax (*Linum* spp.) in a Controlled Environment

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Abstract. **Perennial flax (*Linum austriacum, Linum perenne*, *L. austriacum*) has a wide range of uses for the horticultural and agronomic markets. Protocols for vegetative propagation culture and production scheduling of perennial flax are needed to advance development of potential ornamental or specialty cut flower (CF) cultivars, specifically for the floriculture market. The purpose of this research was to establish vegetative propagation practices for perennial flax CF selections. Fifteen CF genotypes were tested in four experiments between week 8 of 2022 and week 37 of 2023, using 5-cm cuttings from greenhouse (GH) or field cuttings. We tested mist house (MH) rooting time (2 or 3 weeks) and rooting hormone concentrations [1000 or 2000 ppm indole-3-butyric acid (IBA)]. Experiments tested rooting time over 3 or 4 total weeks, which included 2 or 3 weeks under mist, respectively, and 1 week in GH culture before rating. Rooting success was rated using a six-point Likert scale to gauge number and length of roots per cutting. Expt. 1 rooted for 3 weeks using 1000 ppm IBA, resulting in 66.5% rooting. Expt. 2 (4 weeks, 1000 ppm IBA) had 96.4% rooting. Experiment 3 (4 weeks, 2000 ppm IBA) had 82.0% rooting. Expt. 4 (replicate of Expt. 2 but rated using a binary rooted vs. unrooted rating scale, as done in a commercial setting), resulted in 85.4% rooting. Overall, Expts. 2 and 4 resulted in the greatest mean success rates, despite the differences in root rating methods. An increased IBA concentration is not recommended for *Linum* genotypes, as it decreased rooting success in this study. Perennial flax vegetative propagation may be successful using 1000 ppm IBA for 4 weeks to multiply GH stock plants and produce plugs (liners) for shipping and CF production. Additional research will be needed to define the timing and cultural requirements for successful shipping of vernalized, finished (rooted, robust growth) plugs for perennial CF flax cultivars.**

The local and global importance of specialty CFs in the floriculture industry continues to grow, and it is vital to continually introduce new species and cultivars, especially if they can be produced in an environmentally friendly way (Darras 2020; Lan et al. 2022; Salachna 2022). Vegetative or asexual propagation of ornamental crops allows for swift introduction and commercialization of cultivated species (Erwin 2020) and perennial flax may be an ideal candidate (Anderson et al. 2023; Tork et al. 2019). Blue-flowered perennial flax is of interest for ornamental use as both garden bedding plant and CF crop, and ideotype-guided breeding efforts have been under way to develop specific cultivars (Anderson et al. 2023; Tork et al. 2019, 2022c). Blue inflorescences are in high demand due to their rarity as CFs (Dyer et al. 2021; Newsome et al. 2014; Noda 2018). Several blue-flowered flax cultivars exist on the market for landscape or meadow plants (e.g., *L. perenne* 'Blue Flax', 'Sapphire'), although blooms are short-lived and fall each afternoon (Tork et al. 2019). Perennial *Linum* selections are also being studied in development of oilseed and fiber cultivars for regenerative agricultural systems [Ecotone Analytics et al. 2023; Tork et al. 2023; Wyse and Forever Green Initiative (FGI) 2020], pollinator support and honey production (Anderson 2022; Erickson et al. 2021; Ogle et al. 2019), and ecological restoration plantings (Innes et al. 2022; Ogle 2002; Pendleton et al. 2008). Vegetative propagation requirements for perennial flax are understudied and minimal production information exists, which mainly includes sexual propagation from seed, such as for *L. perenne* 'Blue Sapphire' plug production (Ball Horticultural Company 2023). The use of plant "plugs," whether produced from seed or rooted cuttings, has become the norm for many floricultural crops, and their quality greatly influences the growth and quality of the final crop (Park et al. 2022). For instance, it is vital that annual *Linum* root systems are developed before young plants are pinched, stored, vernalized, or shipped (Perry 1998), and practices for perennial *Linum* also should be established. Best practices for perennial flax asexual propagation need to be established for the specialty crop industry and the floriculture market, and this was a primary rationale for the current study. A second rationale was to test the timing and cultural conditions needed to propagate these specific CF selections and find a general reference range for future work in the CF breeding program. It is known that genotype affects rooting competency (Anderson et al. 2023; Druege et al. 2019), and genotypes displaying greater propagative performance would be preferentially selected to advance in a CF cultivar development program.

The American Horticultural Society's recommendations for vegetative propagation of perennial *Linum* include taking softwood or semiripe cuttings in midspring to summer (Toogood 1999). The use of 1000 ppm IBA on vegetative stem cuttings was previously recommended but not required for *Linum*, with Perry (1998) stating that the lower hormone levels should suffice on soft tissue. Dorrell (1974) successfully propagated 5-cm main stem cuttings of annual flax (*L. usitatissimum)* in perlite within 10 d using 1000 ppm IBA, and found that stem age or type of cutting may be factors in rooting success. They also recommended the use of 1000 ppm IBA because it increased rooting rate and development for annual *Linum* cuttings, compared with no IBA (Dorrell 1974). The 1000 ppm IBA concentration was also effective in rooting perennial flax species, using >5-cm cuttings from both field and GH plants, and these were rooted in foam propagation strips over 5 weeks in the MH, under the same conditions used in our current tests (Tork et al. 2022c). In another trial, >5-cm cuttings from field-grown perennial *Linum* species rooted successfully over 6 weeks, although the use of any rooting hormone, type of germination medium, and time in the MH or GH were not reported (Tork et al. 2022b). Vegetative stem tip cuttings of >5 cm, unpinched, with the lower half defoliated and dipped into rooting hormone are the standard protocols for this crop (Tork et al. 2022a, 2022b, 2023), and used in the current research. Additional research recommends the use of synthetic auxins in the form of IBA to root and propagate *Linum* cuttings (Erwin 2020; Murase et al. 2015). Based on these results, 1000 ppm IBA has been shown to be effective in rooting perennial flax, although it is not known if cuttings can be rooted sooner, how they perform in germination mix, whether higher concentrations of IBA affect rooting, and if cuttings sourced from GH- vs. field-grown stock plants vary in performance. The objectives of this research were to test vegetative propagation protocols for rooting time and

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rooting hormone concentration for 15 selected perennial flax CF genotypes.

Materials and Methods

Germplasm

The germplasm used in these trials included *L. perenne, L. austriacum, L. lewisii, L. bienne,* and interspecific hybrids of these two species (Table 1). A total of 15 genotypes were selected and tested for rooting success in all four experiments, and data were recorded for total cuttings obtained. Vegetative propagative protocols for perennial *Linum* were adapted from Tork et al. (2022c), using >5 cm terminal stem tip cuttings, unpinched, with the lower defoliated half dipped in 1000 ppm IBA before sticking.

Experimental design

Four experiments were conducted (Table 2): Expt. 1 (1000 ppm IBA, 3-week Likert scale rating, 72-plug trays); Expt. 2 (1000 ppm IBA with GH cuttings, 4-week Likert scale rating, 50-plug trays); Expt. 3 (2000 ppm IBA with field cuttings, 4-week Likert), 50s; and Expt. 4 (1000 ppm IBA, 4-week commercial rating, 50s). Expt. 1 hypothesized that a decreased rooting time may be effective to root *Linum* using the 1000 ppm IBA. We tested whether 5 weeks was necessary for rooting, by testing rooting percent at 3 weeks, and used germination mix in plug trays rather than rooting in foam cubes, as in prior trials (Tork et al. 2022c). Expt. 2 hypothesized that a greater amount of time may increase rooting success, and tested 4 rather than 3 weeks of total rooting time, including MH and GH. Expt. 3 hypothesized that a higher concentration of IBA (2000 ppm) may provide increased rooting success over 4 weeks. Expt. 4 hypothesized that commercial binary rooting ratings (rooted, unrooted) would be similar to Likert scale ratings, at 4 weeks of rooting (Table 2).

Expt. 1. 1000 ppm IBA, 3-week Likert, 50s or 72s. **Propagation**. Stock plants (n = 3/genotype) were grown in the production GH before commencement of the experiment. In week 8 (2022), basal vegetative tip cuttings of 5 to 6 cm were cut from stock plants of the 15 perennial flax CF genotypes (Table 1), harvesting 10 to 15 healthy cuttings per plant, among three or more stock plants per genotype. Hand-held pruners (Fiskars #399240-1003, Raseborg, Finland) were cleaned with 70% (v/v) ethanol between each stock plant. The propagation protocol used in all experiments selected vigorous, vegetative cuttings of a final 5-cm length with the lower 2.5 cm manually defoliated. Leaf nodes were not counted at the time of cutting harvest, but these genotypes have an average of four or more nodes per cm of stem length at mature vegetative to flowering stage (Goodman et al. 2025), and even more nodes per centimeter at the early vegetative stage, providing ~20 nodes/cutting. Total cuttings tested across genotypes were N = 992, including n = 50 (CF2-CF5) or n = 72 (CF1, CF6-CF15) per genotype, depending on cutting availability on the stock plants (Table 2). Cuttings were stuck into either a 50- or 72-plug tray (P50V, P72SQD, Landmark Plastic, Akron, OH, USA). Moistened germination mix (BM2 Seed Germination and Propagation Mix; Berger, Saint-Modeste, QC, Canada) was used, and the cut defoliated ends of cuttings were dipped into 1000 ppm IBA (Maia Products, Inc. – Hormex, Westlake Village, CA, USA) before sticking. Unlike previous perennial *Linum* propagation trials using foam strips for 5 weeks (Tork et al. 2022c), this tested germination mix in 50- or 72-plug trays for 3 weeks. Cuttings were under an intermittent mist system in the MH for 2 weeks of rooting (21 ± 1/21 ± 1 °C, day/night, 16 h, 0600 to 2200 HR) high-intensity discharge lighting at a minimum set point of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a mist frequency of 10-min intervals (mist nozzles, reverse osmosis water) during 0600 to 2200 HR with a 7-s duration. Greenhouse production conditions (post-rooting culture) included supplemental lighting supplied by 400-W high-pressure sodium high-intensity discharge (HPS-HID) lamps at a minimum of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level, at 16 ± 1 °C, 16 h day/8 h night photoperiod. Plants were fertigated at a constant liquid feed rate of 125 ppm N from water-soluble 20N–10P–20K fertilizer on weekdays and watered with tap water on weekends. The propagation MH and production GH (double-strength float glass roof, double-wall acrylic sidewalls) for all experiments are located at the Minnesota Agricultural Experiment Station, or MAES (lat. 44°59′17.8″N, long. −93°10′51.6″W) within the Plant Growth Facilities on the University of Minnesota, St. Paul, MN, campus. These locations and conditions were identical to those used by Tork et al. (2022c) in all propagation trials as well.

After 1 week of GH growth, root ratings were done using a six-point Likert scale to classify rooting success after 3 total weeks. Ratings were conducted by removing each rooted cutting from the plug tray using a long plant tag, gently tapping substrate off of roots, and laying each cutting down on a black plastic tray to measure root length (cm) and determine ratings (Likert scale). Likert scale ratings of zero were ranked as unsuccessful, whereas ≥1 to 5 ratings were successful for rooting; and lengths (cm) refer to root length. Likert scale rating phenotypes were as follows (adapted from Anderson et al. 2016): 0 = no roots nor callus visible; 1 = ≤0.5-cm root(s) and/or 1 to 2 roots, callus may be present; 2 = 0.5 cm–1.0 cm and/or 3 to 4 roots; 3 = ≤1.0 cm and/or 5 to 6 roots; 4 = ≥1.0 cm

Table 1. Advanced perennial flax cut flower (CF) genotypes (CF1 to CF15), *Linum* taxa, and initial selection attributes for certain ideotypes (Tork et al. 2019).

Genotype	Confirmed *Linum* taxa after SNP analyses[i]	Original *Linum* taxonomic designations	Selection attributes[ii]
CF1	Undetermined; assumed to be *Linum perenne*	*Linum perenne* (F_1 hybrid from OS accession)	FL, OS
CF2	*L. perenne*	*L. austriacum* (F_1 hybrid)	CF
CF3	*L. perenne*	*L. austriacum* (F_1 hybrid)	CF, F
CF4	*L. austriacum*	*L. perenne* × *L. austriacum* (F_1 hybrid)	FLM, F
CF5	*L. perenne* × *L. lewisii*	*L. perenne*	CF, OS
CF6	*L. austriacum*	*L. perenne* (F_1 hybrid)	CF
CF7	Undetermined; assumed to be *L. perenne* × *L. perenne*	*L. perenne* × CF3/*L. austriacum* (F_1 hybrid)	CF, FL
CF8	*L. austriacum*	*L. perenne* × *L. perenne* (F_1 hybrid)	CF
CF9	*L. lewisii*	*L. perenne* × CF16/*L. perenne* (F_1 hybrid)	CF, OS
CF10	*L. bienne*	*L. perenne*	CF, OS
CF11	Undetermined; assumed *L. perenne*	*L. perenne*	CF, OS, R, LR
CF12	*L. austriacum* × *L. perenne*	*L. perenne*	CF, OS, R, LR
CF13	Not tested	*L. perenne*	FL, R, OS
CF14	*L. austriacum*	*L. perenne*	R, OS
CF15	*L. austriacum*	*L. perenne*	CF, FL, OS

[i] Single nucleotide polymorphisms (SNPs) analyses conducted by DArTseq (Abbey and Anderson, 2024, unpublished data).
[ii] CF = cut flower, F = fiber, FL = floriferous, FLM = floral markings, LR = lodging resistance, R = regrowth potential, OS = oilseed.

Table 2. Vegetative propagation experiments and factors, including rooting hormone (indole-3-butyric acid, IBA) concentration, rooting time (number of weeks), source of cuttings (GH = greenhouse; field), plug tray size used (50, 72), and sample size (number of cutting replications per treatment), for 15 genotypes of perennial cut flower (CF) flax (*cf.* Table 1).

Expt.	Rooting hormone, IBA (ppm)	Rooting time (no. wk)	Cutting source (GH, field)	Plug tray size (no. plugs / tray)	Sample size (no. cutting reps / treatment)
Expt. 1	1000	3	GH	72	n = 992
Expt. 2	1000	4	GH	50	n = 720
Expt. 3	2000	4	Field	50	n = 150
Expt. 4	1000	4	GH	50	n = 315

Fig. 1. Root ratings (six-point Likert scale) of perennial flax cuttings (5 cm) used in Expt. 1, after 3 weeks of total rooting time using 1000 ppm indole-3-butyric acid (IBA), and germination mix in 72-plug trays. Cut flower flax genotypes CF7 (**A**) and CF15 (**B**) demonstrate various root lengths, rooting success ratings, and presence or lack of tip necrosis. Likert scale ratings: 0 = no roots or callus visible; 1 = ≤0.5-cm root and/or 1 to 2 roots, root callus may be present; 2 = 0.5 cm to 1.0 cm and/or 3 to 4 roots; 3 = ≤1.0 cm and/or 5 to 6 roots; 4 = ≥1.0 cm and/or 7 to 8 roots; 5 = >1.0 cm and/or 9+ roots. Scale bar = 5 cm. Photo credits: Elizabeth Goodman.

and/or 7 to 8 roots; 5 = >1.0 cm and/or 9+ roots (Fig. 1A and B). Once ratings were tallied, a digital photograph was taken, rooted cuttings were replanted into 50-plug trays (P50V; Landmark Plastic), terminal (apical) meristems were soft pinched, and plugs were grown-on for 10 weeks for field transplanting.

Expt. 2. 1000 ppm IBA, 4-week Likert, 50s. Propagation methods used, other than rooting time and plug tray size, were identical to Expt. 1. A total of n = 48 cuttings/genotype (N = 720) were treated with 1000 ppm IBA and stuck in week 30 (2022; Table 2) in 50-plug trays (P50V, Landmark Plastic). The larger plug tray size was chosen after observing roots filling the 72-plug volume quickly in Expt. 1. Soilless medium surfaces were also dibbled (holes poked into substrate to stick cuttings into) to facilitate ease of cutting insertion in the plugs and to minimize stem damage or occlusion (Lionakis Meyer 2004). Cuttings were rooted for 3 weeks in the MH and grown-on for 1 week in the GH before roots were rated at the final 4 weeks using the Likert scale (Expt. 1).

Expt. 3. 2000 ppm IBA, 4-week Likert, field cuttings, 50s. Cuttings were harvested and stuck the same day in week 33 (2022) with n = 10 cuttings/genotype (N = 150; Table 2), using the same harvesting protocol and pruner as in the GH. Harvest occurred during the second year of growth from the St. Paul field (Expt. 1 plants), located at MAES. The field had N = 300 plants spaced 45.7 cm on center within rows, and rows were spaced 2.1 m apart. Weekly field irrigation of 2.54 cm was provided if rainfall was insufficient, and 50 lb/acre of N as urea was applied for the season. Weed control consisted of weekly hand-hoeing, monthly mechanical tillage between rows, and pre-emergent herbicide applications (Fortress, OHP Inc., Bluffton, SC, USA) at the recommended rates. Upon harvest, the 5- to 6-cm cuttings were wrapped in a wet (tap water) paper towel inside a zippered plastic bag, transported from field to laboratory in a cooler (~10 °C), using an ice pack covered with a fabric towel. Cuttings were rooted in 2000 ppm IBA with a 4-week rooting period in 50-plug trays, using Expt. 2 propagation protocol. Specifically, cuttings were rooted for 3 weeks in the MH and grown-on for 1 week in the GH before roots were rated using the Likert scale at a total of 4 weeks.

Expt. 4. 1000 ppm IBA, 4-week commercial, 50s. This experiment commenced in week 49 (2022) to assess whether rooting success using commercial root ratings (rooted/unrooted) corresponded to Likert ratings. All propagation methods and materials were identical to Expt. 2, except for the rating method. Rooted cuttings (N = 315, n = 21/genotype/treatment; Table 2) were rated (binary scale) for successful/non-successful rooting after 4 weeks. If cuttings resisted being pulled from the plugs, they were rated "successful," whereas a lack of resistance and cuttings removal indicated no root development, and were then rated as "non-successful." Any resistance indicated rooting, although this was not quantified. Any resistance indicated rooting, even if roots were torn off. This rating system more closely matches commercial production techniques at rooting or sticking stations (Anderson 2007), and its efficacy should be known.

Data analyses

Rooting data were collected in Expts. 1, 2, and 3 as percent success of cuttings rooted per genotype, based on a six-point Likert scale to quantify root number and length per cutting. The percent rooting ("success") included ratings ≥1 and excluded ratings = 0 of no roots or callus present. Likert scale classifications were: 0 = no roots nor callus visible; 1 = ≤0.5 cm and/or 1 to 2 roots, callus may be present; 2 = 0.5 cm–1.0 cm and/or 3 to 4 roots; 3 = ≤1.0 cm and/or 5 to 6 roots; 4 = ≥1.0 cm and/or 7 to 8 roots; 5 = >1.0 cm and/or 9+ roots. To determine if rooting success was equally distributed across Likert rankings within genotypes, χ^2 tests (1:1:1:1:1:1 χ^2, df = 5) were performed in Expts. 1, 2, and 3 (critical χ^2 value >11.07), and a (1:1 χ^2, df = 1) χ^2 was performed for Expt. 4 rooting data as a binary rating (critical χ^2 value >3.84). To normalize these data, the percent rooting data were transformed using arcsine [SQRT(rooting %)*ASIN(rooting %)] transformation. Two-tailed *t* tests ($\alpha = 0.05$) were also performed for all four experiments to determine differences among genotypes. Because data analysis in Expt. 4 did not include the Likert scale ratings, the transformed binary rooting ratings were used for the *t* tests.

Results

Expt. 1. A significant genotype interaction was observed for rooting performance over 3 weeks (Table 3). Percent rooting for the 3-week propagation period ranged from 12% (CF3) to 100% (CF11, CF13, CF15) (Table 3), and had a grand mean of 66.5% across genotypes. Five genotypes had <50% successful rooting (CF1, CF3, CF4, CF6, CF10) and the remaining exceeded 50% rooting. The highest (100%) rooting percent occurred in genotypes CF11, CF13, and CF15, which also had no 0-ratings. The next-highest rooting percent was with CF14 (97.2%), CF8 (90.3%), and CF12 (86.1%) (Table 3).

χ^2 tests (1:1:1:1:1:1 χ^2, df = 5) showed that all genotypes except CF8 (χ^2 = 7.83) and CF9 (χ^2 = 11.33) had significantly different rooting distributions at the $P \leq 0.001$ level. The χ^2 of greatest significance ranged from low-rooting CF6 (χ^2 = 261.17) to high-rooting CF15 (χ^2 = 212.83), as these two deviated the furthest from an equal distribution (Table 3). A *t* test distribution (two-tailed) showed a significant ($P \leq 0.001$) difference between genotypes for rooting percentage at $t = 11.88$ (data not shown). Despite using vegetative cuttings as propagules, several genotypes (CF3, CF4, CF9, CF10, CF12, CF13) had flower bud initiation and development before the root rating (Fig. 2). This differentiation into flowering did not seem to decrease or correlate with rooting performance, although this was not directly tested. Additional phenotypic observations included meristematic tip necrosis (CF2, CF3, CF4, CF6, CF7, CF10), leaf chlorosis (CF2, CF9), cut end stem plugging and necrosis with tissue desiccation (CF6), and/or increased anthocyanin pigmentation (CF7, CF8).

Expt. 2. The total number of cuttings stuck was N = 720, n = 48/genotype (Table 2), and the mean rooting across genotypes was

Table 3. Root ratings (0 to 5) at 3 weeks for 15 genotypes (CF1–CF15) of perennial cut flower flax cuttings (5 cm) rooted with 1000 ppm indole-3-butyric acid (IBA) in 50- or 72-plug trays. Successful rooting percent included ratings ≥1 through 5 and excluded ratings = 0 of no roots nor callus present. χ^2 tests (df = 5) performed to determine equal distribution of rooting across ratings for N = 992 cuttings, n = 50 to 72/genotype.

	No. of rated cuttings							
Genotype	Rating = 0	Rating = 1	Rating = 2	Rating = 3	Rating = 4	Rating = 5	% rooting (≥1 rating)	1:1:1:1:1:1 χ^2 value
CF1	40	20	2	2	1	7	44.4	99.50***
CF2	10	4	0	0	0	36	80.4	119.75***
CF3	44	2	1	2	1	0	12.0	183.52***
CF4	27	6	4	2	4	7	46.0	52.00***
CF5	24	3	3	4	1	15	52.0	50.32***
CF6	63	1	1	1	1	5	12.5	261.17***
CF7	27	18	8	3	9	7	62.5	32.67***
CF8	7	14	14	6	15	16	90.3	7.83^{NS}
CF9	12	16	10	6	8	20	83.3	11.33*
CF10	50	7	3	4	3	5	30.6	145.33***
CF11	0	3	2	3	12	52	100.0	167.17***
CF12	10	7	3	0	20	32	86.1	59.83***
CF13	0	3	2	5	23	39	100.0	102.00***
CF14	2	4	1	0	16	49	97.2	151.17***
CF15	0	2	4	3	5	58	100.0	212.83***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

96.4%, or ≈30% higher than the 3-week rooting mean (Expt. 1). Root ratings were not equally distributed among genotypes after the 4-week rooting time, based on χ^2 tests (1:1:1:1:1:1 χ^2, df = 5), and all but CF12 had significantly more 5-ratings than in Expt. 1 (Table 4). The rooting percentages ranged from 77.1% for CF12 to 100% for CF3, CF6, CF7, CF11, and CF13. All genotype root ratings had significant χ^2 values at the ($P \leq 0.001$) level (Table 4). A two-tailed t test ($t = 12.36$) showed significant ($P \leq 0.001$) differences among genotypes for rooting percentage (data not shown), as in Expt. 1.

Expt. 3. Genotype rooting responses for total cuttings of N = 150, n = 10/genotype with 2000 ppm IBA were not comparable (to trials using 1000 ppm IBA) in this experiment (Table 5). The lowest rooting percentages (40%, 50%) were in CF13 and CF12, respectively, and the highest percentages (100%) occurred in CF7, CF10, CF14, and CF15 (Table 5). Genotype Likert scale root ratings had a wide distribution range of significant 1:1:1:1:1:1 χ^2 values in Expt. 3. Specifically, seven genotypes were significant at the $P \leq 0.001$ level (CF1, CF3, CF5, CF10, CF11, CF14, CF15), and five were significant at the $P \leq 0.01$ level (CF6-CF9, CF13) (Table 5). Genotype root ratings that did not result in a significant χ^2 included CF2 (4.40), CF4 (10.40), and CF12 (8.00) (Table 5). A two-tailed t test ($t = 11.75$) showed significant ($P \leq 0.001$) differences among genotypes for rooting percentage (data not shown). Similar to results of Expt. 2, genotypes CF7, CF10, CF14, and CF15 had the highest rooting response ratings.

Expt. 4. Genotype rooting responses under 1000 ppm IBA over 4 weeks in 50-plug trays using a commercial, binary testing method ranged from 61.9% (CF3) to 100% (CF5, CF10, CF13) for total cuttings of N = 315, n = 21 (Table 6). This experiment resulted in a grand mean of 85.4% rooting across genotypes, intermediate between results of Expts. 2 and 3. χ^2 tests (1:1 χ^2, df = 1) were performed to determine equal distribution of rooting across genotypes, and the critical χ^2 value was >3.84 for $\alpha = 0.05$ (Table 6). Eight genotypes had >90% rooting (CF4, CF5, CF7, CF10, CF11, CF12, CF13, CF14), and these also had a χ^2 significant at $P \leq 0.001$. For rooting competency, three genotypes were significant at the $P \leq 0.01$ level (CF1, CF2, CF6), one genotype at the $P \leq 0.05$ level (CF8), and three genotypes that did not result in a significant χ^2 included CF3 (1.19), CF9 (2.33), and CF15 (2.33) (Table 7). The two-tailed t test ($t = 12.60$) was significant ($P \leq 0.001$) at the $\alpha = 0.05$ level (data not shown).

Fig. 2. Rooted 72-plug tray of 5-cm perennial flax cut flower genotype (CF13) vegetative cuttings 7 weeks after sticking (Expt. 1). Note the tall cuttings, with some budding and flowering left of center. Scale bar = 5 cm. Photo credit Elizabeth Goodman.

Discussion

In Expt. 1, the 66.5% grand mean rooting success would be generally unacceptable for commercial propagation (Anderson et al. 2016). Thus, the Expt. 1 methods are not recommended, and we must reject the null hypothesis that 3 weeks is sufficient rooting time for perennial flax. This initial trial run was intended to establish propagation protocols, and test the shortest rooting time of 3 weeks, and these factors may have decreased the overall rooting success rates. Observations of meristematic tip necrosis, cut end stem plugging, and leaf chlorosis and necrosis in this trial informed the use of dibbling in subsequent Expts. 2 to 4. Genotype-dependent phenotypic expression is expected in production, and this was observed in Expt. 1 as a wide range of rooting percentages and differences in phenology. Several variables such as photoperiod, light quality (e.g., red to far-red ratio), stress response, and unique genotypic expression will affect flowering (Bergstrand 2017; Erwin 2020). It is not uncommon for young, unpinched perennial plants to flower sooner than pinched plants, as seen with *Veronica spicata* 'Red Fox'; and producers may pinch rooted cuttings to bulk up transplant plugs, to vernalize, or to ship out (Enfield 2002). In this initial test, the factor of rooting time likely had a significant effect on overall rooting performance, although the effect of genotype produced the wide range of rooting percentages seen (Druege et al. 2019) among the 15 CF genotypes.

In Expt. 2, mean rooting percent across genotypes was greatest at 96.4%, which was ~30% higher than the 3-week rooting mean in Expt. 1. Expt. 2 increased the MH time by 1 week, for a total 4-week rooting time with 1000 ppm IBA, and in 50-plug trays.

Table 4. Root ratings (0 to 5) at 4 weeks for 15 genotypes (CF1-CF15) of perennial cut flower flax cuttings (5 cm) rooted with 1000 ppm indole-3-butyric acid (IBA) in 50-plug trays. Successful rooting percent included ratings ≥1 through 5 and excluded ratings = 0 of no roots nor callus present. χ^2 tests (df = 5) performed to determine equal distribution of rooting across ratings for N = 720 cuttings, n = 48/genotype.

	No. of rated cuttings							
Genotype	Rating = 0	Rating = 1	Rating = 2	Rating = 3	Rating = 4	Rating = 5	% rooting (≥1 rating)	1:1:1:1:1:1:1 χ^2 value
CF1	4	2	1	1	2	38	91.7	135.75***
CF2	1	1	6	2	4	34	97.9	103.75***
CF3	0	0	0	2	2	44	100.0	195.00***
CF4	1	0	0	2	5	40	97.9	155.75***
CF5	1	0	0	0	1	46	97.9	216.75***
CF6	0	0	0	0	1	47	100.0	228.25***
CF7	0	0	1	1	4	42	100.0	174.75***
CF8	3	0	0	2	4	39	93.8	145.75***
CF9	1	1	0	0	0	46	97.9	216.75***
CF10	2	1	0	0	1	44	95.8	194.75***
CF11	0	1	1	0	0	46	100.0	216.75***
CF12	11	6	1	3	1	26	77.1	57.50***
CF13	0	0	0	0	3	45	100.0	206.25***
CF14	1	1	0	0	1	45	97.9	205.50***
CF15	1	0	0	0	0	47	97.9	228.25***

***Significant at $P \leq 0.001$.

Table 5. Root ratings (0 to 5) at 4 weeks for 15 genotypes (CF1-CF15) of perennial cut flower flax cuttings (5 cm) rooted with 2000 ppm indole-3-butyric acid (IBA) in 50-plug trays. Successful rooting percentages included ratings ≥1 through 5 and excluded ratings = 0 of no roots nor callus present. χ^2 tests (df = 5) performed to determine equal distribution of rooting across ratings for N = 150 cuttings, n = 10/genotype.

	No. of rated cuttings							
Genotype	Rating = 0	Rating = 1	Rating = 2	Rating = 3	Rating = 4	Rating = 5	% rooting (≥1 rating)	1:1:1:1:1:1:1 χ^2 value
CF1	2	0	0	0	0	8	80.0	30.80***
CF2	3	2	1	0	3	1	70.0	4.40^{NS}
CF3	2	1	0	0	0	7	80.0	22.40***
CF4	2	2	0	0	1	5	80.0	10.40^{NS}
CF5	1	0	0	0	0	9	90.0	39.20***
CF6	1	1	0	0	2	6	90.0	15.20**
CF7	0	0	0	1	5	4	100.0	15.20**
CF8	1	2	0	0	1	6	90.0	15.20**
CF9	3	1	0	0	0	6	70.0	15.20**
CF10	0	0	0	0	0	10	100.0	50.00***
CF11	1	0	0	0	1	8	90.0	29.60***
CF12	5	1	1	1	1	1	50.0	8.00^{NS}
CF13	6	1	2	1	0	0	40.0	15.20**
CF14	0	1	0	0	1	8	100.0	29.60***
CF15	2	0	0	0	0	8	100.0	29.60***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Genotypes that had <50% rooting in Expt. 1 (CF1, CF3, CF4, CF6, CF10) had >90% rooting in Expt. 2. The substantial gain in root development in only a week exemplifies the importance of testing rooting time length and plug tray size to establish effective propagation parameters. We fail to reject the null hypothesis that increased rooting time will increase rooting success percentages. The floriculture industry uses crop scheduling and requires uniformity of plug trays, so an exact amount of time to "finishing" (rooted with a well-developed root system; ready for commercial sale) is vital for all GH crops (Lionakis Meyer 2004; Perry 1998). The *Linum lewisii* perennial flax species has also been propagated in ∼4 weeks under a 16-h photoperiod in GH culture (Gossweiler et al. 2024), so this may be a reliable general rooting time for perennial *Linum* species. Some genotypes with the greatest rooting percentages in Expt. 1 also had similarly high rooting percentages in Expt. 2, and included CF11, CF13, CF14, and CF15. This may indicate a greater propagative ability in these genotypes, and is a positive attribute when developing cultivars for commercial production (Anderson 2007; Erwin 2020; Lionakis Meyer 2004). Meristematic tip necrosis was observed on seven genotypes (CF1, CF2, CF3, CF5, CF6, CF10, CF12) in this trial as well, although stem plugging was not, which was likely affected by dibbling before sticking in Expt. 2. Increased rooting success may

Table 6. Root ratings (binary 0 or 1) at 4 weeks for 15 genotypes (CF1–CF15) of perennial cut flower flax cuttings (5 cm) rooted with 1000 ppm indole-3-butyric acid (IBA). Binary rooting percentage excluded cuttings with no roots present (unsuccessfully rooted). χ^2 tests (df = 1) performed to determine equal distribution of rooting across genotypes for N = 315 cuttings, n = 21/genotype.

Genotype	No. successfully rooted	No. Unsuccessfully rooted	% rooted	1:1 χ^2 value
CF1	18	3	85.7	10.71**
CF2	17	4	81.0	8.05**
CF3	13	8	61.9	1.19^{NS}
CF4	19	2	90.5	13.76***
CF5	21	0	100.0	21.00***
CF6	18	3	85.7	10.71**
CF7	20	1	95.2	17.19***
CF8	16	5	76.2	5.76*
CF9	14	7	66.7	2.33^{NS}
CF10	21	0	100.0	21.00***
CF11	19	2	90.5	13.76***
CF12	19	2	90.5	13.76***
CF13	21	0	100.0	21.00***
CF14	19	2	90.5	13.76***
CF15	14	7	66.7	2.33^{NS}

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 7. Comparison of 3- and 4-week root ratings of perennial cut flower flax cuttings (5 cm), by indole-3-butyric acid (IBA) concentration of 1000 ppm (N = 992, N = 720, N = 315) and 2000 ppm (N = 150). χ^2 tests (1:1:1:1:1:1 χ^2, df = 5; 1:1 χ^2, df = 1) performed to determine equal distribution of rooting across 15 genotypes. Expts. 1, 2, and 3 rated using six-point Likert scale, Expt. 4 only used binary rooting percentage assessments, with ratings ≥ 1 and excludes ratings = 0 of no roots or callus present.

	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	3-wk rooting 1000 ppm IBA (N = 992)		4-wk rooting 1000 ppm IBA (N = 720)		4-wk rooting 2000 ppm IBA (N = 150)		4-wk rooting 1000 ppm IBA (N = 315)	
Genotype	1:1:1:1:1:1 χ^2	% rooting	1:1:1:1:1:1 χ^2	% rooting	1:1:1:1:1:1 χ^2	% rooting	1:1 χ^2	% rooting
CF1	99.50***	44.4	135.75***	91.7	30.80***	80.0	10.71**	85.7
CF2	119.75***	80.4	103.75***	97.9	4.40^{NS}	70.0	8.05**	81.0
CF3	183.52***	12.0	195.00***	100.0	22.40***	80.0	1.19^{NS}	61.9
CF4	52.00***	46.0	155.75***	97.9	10.40^{NS}	80.0	13.76***	90.5
CF5	50.32***	52.0	216.75***	97.9	39.20***	90.0	21.00***	100.0
CF6	261.17***	12.5	228.25***	100.0	15.20**	90.0	10.71**	85.7
CF7	32.67***	62.5	174.75***	100.0	15.20**	100.0	17.19***	95.2
CF8	7.83^{NS}	90.3	145.75***	93.8	15.20**	90.0	5.76*	76.2
CF9	11.33*	83.3	216.75***	97.9	15.20**	70.0	2.33^{NS}	66.7
CF10	145.33***	30.6	194.75***	95.8	50.00***	100.0	21.00***	100.0
CF11	167.17***	100.0	216.75***	100.0	29.60***	90.0	13.76***	90.5
CF12	59.83***	86.1	57.50***	77.1	8.00^{NS}	50.0	13.76***	90.5
CF13	102.00***	100.0	206.25***	100.0	15.20**	40.0	21.00***	100.0
CF14	151.17***	97.2	205.50***	97.9	29.60***	100.0	13.76***	90.5
CF15	212.83***	100.0	228.25***	97.9	29.60***	100.0	2.33^{NS}	66.7
Mean rooting %	66.5		96.4		82.0		85.4	

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

have been affected by the refined propagation methods, including dibbling, to minimize stem xylem plugging (Lionakis Meyer 2004). Adventitious rooting from a single fallen leaf was observed with both CF4 and CF10, which had not been reported in previous literature (Druege et al. 2019), and may be indicative of the propagative ability of perennial *Linum* species.

Expt. 3 tested field cuttings rooted in 2000 ppm IBA for 4 weeks and resulted in an 82.0% mean success rating across genotypes. Previously "successful" genotypes had much lower rooting percentages, such as CF12 (50.0%) and CF13 (40.0%), CF2, CF9 (70.0%). Alternately, genotypes that had low rooting percentages in Expt. 1 and increased rooting percentages in Expt. 2 (CF1, CF3, CF4) had intermediate results of 80.0% under Expt. 3 treatments (Table 4). Compared with results from Expts. 1 and 2, doubling the IBA concentration resulted in a decrease of ∼14% from the 1000 ppm treatment for 4 weeks. Thus, the length of rooting time likely had a greater positive impact on rooting success than hormone concentration for all trials, despite the field-sourced cuttings being a confounding variable. The Expt. 2 Likert ratings were spread more consistently to the higher end of the scale than in Expt. 3, and Expt. 2 also had more replication. The null hypothesis, that a greater level of IBA would increase rooting success over 4 weeks, compared with the lower level of hormone for that same propagation time, is rejected. Some perennials may not respond to plant growth regulators such as an IBA rooting hormone, and may respond more to light quantity, a change in temperature, or increased rooting time (Enfield 2002; Erwin 2020; Park et al. 2022). Although, in propagating CF7, CF10, CF14, and CF15, higher IBA concentrations may be effective due to genotype response, stem cutting position, or both (Anderson et al. 2016; Dorrell 1974). GH-grown cuttings could have been tested with the 2000 ppm IBA to have a replication of Expt. 3, and/or field-grown cuttings tested with the 1000 ppm IBA, but prior trials successfully propagated cuttings from both sources using 1000 ppm IBA (Tork et al. 2022c), so additional tests of IBA concentration were not conducted. Propagation and production of flowering herbaceous perennials is not as simple as many annual or cultivated ornamental perennial species, due to these types of unique requirements (Nordwig and Erwin 1999).

Experiment 4 rooting success rates ranged from 61.9% to 100% and had a grand mean of 85.4%, which is ∼10% lower than this same treatment in Expt. 2, which used the Likert rating scale for rooting percentage. This binary rating method still resulted in unequal distribution of rooting percentage, as Expts. 1 to 3 had. The 85.4% grand rooting mean across genotypes in Expt. 4 was lower than in Expt. 2 (96.4%), and higher than the Expt. 3 results (82.0%). We fail to reject the null hypothesis that the binary commercial root rating is comparable to the Likert scale. The χ^2 test (1:1 χ^2, df = 1) also resulted in unequal distribution of rooting performance among genotypes. Eight genotypes had a χ^2 significant at $P \leq 0.001$, three genotypes were significant at the $P \leq 0.01$ level, one genotype at the $P \leq 0.05$ level, and three genotypes did not result in a significant χ^2. The two-tailed t test ($t = 12.60$) was significant ($P \leq 0.001$) at the $\alpha = 0.05$ level (data not shown).

In comparing all four experimental results, despite the type of rating system used, these 15 genotypes rooted significantly better with 1000 ppm IBA in a 4-week rooting time, that is, Expts. 2 and 4 (Table 7). In all four experiments, two-tailed t tests resulted in significantly different rooting distributions among genotypes. This reaffirms the importance of genetic control of phenotypic expression during production, even at the propagative cutting stage (Park et al. 2022). Genotypic differences persist through the propagation phase into production and postharvest (Anderson 2007; Diederichsen 2019; Erwin 2020), and this is also observed with the *Linum* CF genotypes. This research supports the use of 1000 ppm IBA for perennial *Linum* vegetative propagation, as a higher concentration did not improve rooting percentage, and the 96.4% mean rooting success rate is acceptable for industry (Anderson et al. 2016; Erwin 2020). It is noteworthy that some genotypes consistently performed highest across variables, such as CF11 and CF14, although many others also showed acceptable propagative ability (Table 6). The genotypes did not fit an equal distribution for χ^2 tests and significant differences in rooting performance were observed within and among genotypes. A wide distribution of rooting performance among selected perennial *Linum* hybrids was expected, as wild traits were still present and may indicate a similar distribution across other phenotypic traits, such as shoot growth (Anderson et al. 2023). The genotype × environment interaction effect on phenotypic expression is important to consider with all species, especially *Linum* (Brutch et al. 2020; Diederichsen 2019; Gossweiler et al. 2024). Phenotype is also affected by the quality and quantity of light, measured by photosynthetic photon flux density (PPFD), often set at specific levels for propagation and production of certain species. Many ornamental floriculture species may form more root and shoot mass under higher PPFD during propagation, whether annual or perennial, or soft tissue or hardwood cuttings (Park et al. 2022). The 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD used for both MH and GH in these trials was considered sufficient, and had been previously used successfully (Tork et al. 2022c). The 4-week timeframe under a 16-h photoperiod of 100 to 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is typically acceptable for rooting perennial

cuttings (Park et al. 2022). It is possible that *Linum* species may respond to higher levels of irradiance in propagation and production, since species are either obligate long-day plants or day-neutral plants with facultative irradiance (Erwin 2020; Mattson and Erwin, 2005). Determining photoperiodicity for flowering in annual *Linum* has been challenging (Brutch et al. 2020; Domantovich et al. 2012), and determining this for perennial *Linum* species or selections may be necessary for future research.

The 4-week rooting time in the soilless germination medium is an effective protocol for propagating by cuttings rather than the previously used 5 weeks in foam strips (Tork et al. 2022c). We did not directly compare these two methods, as rooting means for the 5-week trial were not reported (Tork et al. 2022c). A 4-week minimum vegetative propagation time for plugs (before they can be pinched, transplanted, or shipped) informs industry production schedules, and can be adjusted for specific cultivars as needed. Only in Expt. 4 were the rooted cuttings not pinched after being rated, and many flowered earlier and from side branches rather than from the main stem. Based on these results, it may be beneficial to soft pinch the cuttings when used for stock plant production, but not for CF production. Most genotypes bulked up quickly if evenly watered, and became rootbound when grown for ≥10 weeks in 50-plug trays, indicating that 10 weeks was the maximum duration of plug stage before transplanting, and it was beyond the "finished" stage. This information guides future perennial flax propagation and production scheduling, particularly if 50-plug tray rooted cuttings are vernalized over the winter for spring sales (Nordwig and Erwin 1999; Perry 1998). Best practices to obtain maximum rooting success rates are essential to ensure commercial success of marketed cultivars (Bergstrand 2017; Markovic et al. 2020). The horticulture and floriculture industries require healthy, viable stock plants to serve as sources for asexual propagule generation (Erwin 2020; Faust 2020). Future studies should also establish suitable stock plant production and maintenance parameters for these perennial *Linum* genotypes, considering the drought- and heat-tolerance of these species. Stock plants used to provide cuttings were 6-month-old GH-grown plugs planted three per 25.4 cm azalea pot (Fig. 3).

Fig. 3. Greenhouse stock plants (top) and 72-plug tray (bottom) of a perennial flax cut flower genotype (CF1). Rooted vegetative cuttings (5 cm) shown 4 weeks after sticking, after root ratings, taken from greenhouse-grown stock plants at three per 25.4 cm azalea pot. Scale bar = 5 cm. Photo credit Elizabeth Goodman.

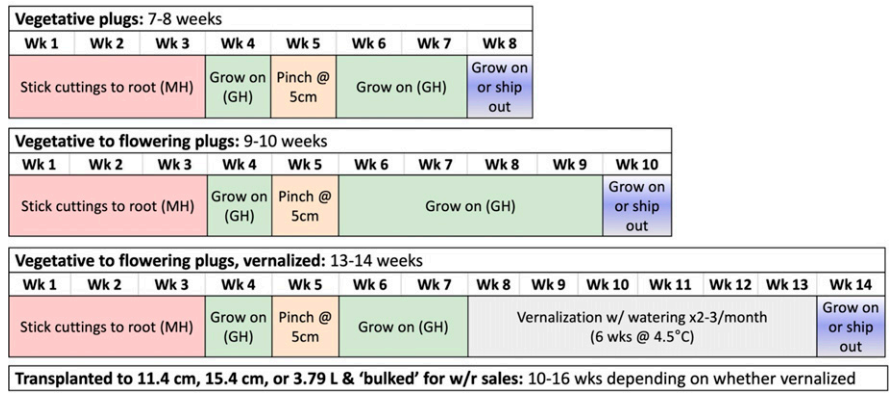

Fig. 4. Potential propagation and early production schedules for vegetative, flowering, and vernalized plugs (liners) of perennial *Linum* species.

Finished vegetative plugs can be transplanted or shipped at 7 to 8 weeks, and rooting or flowering may vary by genotype (Fig. 4). Finished vegetative to flowering plugs can be transplanted or shipped at 9 to 10 weeks. Plugs should be transplanted at a maximum of 9 to 10 weeks after sticking, to avoid root disturbance or rootbound plugs, unless vernalizing. After the 7- to 8-week vegetative plug stage, plugs may be vernalized for ≥6 weeks at 4.5 °C and watered two to three times/month, or as needed per substrate volume (Fig. 4). *Linum* does not tolerate saturated substrates, and allowing plants or large plugs to dry out somewhat between irrigation is needed (Ball Horticultural Company 2023; Hanchek 2021). The sample schedule of 13 to 14 weeks for vernalized, flowering plugs can be used as a guide to establish more refined propagation and production schedules for cultivars, as pinching and shipping times will vary depending on genotypic performance (Fig. 4). Finished rooted plugs can be transplanted into 11.4-cm, 15.4-cm, or 3.79-L containers and "bulked" for wholesale or retail sales in 10 to 16 weeks, depending on vernalization or not. A crop schedule or production guide is valuable information for producers, as it assists them in planning and budgeting (Fig. 4). Perennial flax vegetative propagation using this protocol will generate rooted propagules for commercial producers, ranging from vegetative plugs to vernalized, flowering potted plants for wholesale or retail companies.

Conclusions

Asexual propagation of perennial flax recommendations are to use 1000 ppm IBA on the defoliated half of a 5-cm vegetative cutting and rooted (in dibbled, moistened germination mix) for a total duration of ≥4 weeks, with a maximum of 2 to 3 weeks in the MH to maximize cutting health. The 2000 ppm IBA concentration is not recommended for rooting *L. perenne* and *L. austriacum*. A growing-on period of 1 to 2 weeks before soft pinching, vernalizing, or shipping is needed to ensure established root systems.

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