Propagation of Selected Clones of Eastern Redbud (*Cercis canadensis*) by Stem Cuttings[1]

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Abstract

Two experiments, one utilizing softwood cuttings and the other semi-hardwood cuttings, were conducted to investigate the influence of growth stage and auxin treatment on rooting four related clones of eastern redbud (*Cercis canadensis* L.). The four clones were 'Flame' (*C. canadensis* L. 'Flame'), dwarf white, and two selections (NC99-6-1 and NC99-6-2) of an F_1 generation derived from a cross of 'Flame' and dwarf white. At each growth stage, rooting responses of the clones varied and were influenced greatly by auxin treatment, demonstrating the variable rooting potential of the genotypes. In both studies, 'Flame' rooted well [63% rooting in the softwood study when treated with the potassium (K) salt (K-salt) of indolebutyric acid (IBA) at 5000 mg·L^{-1} (ppm), 83% rooting when treated in the semi-hardwood study with K-IBA at 10,000 mg·L^{-1}], indicating stem cuttings may be a commercially feasible means of propagation for 'Flame.' Softwood cuttings of dwarf white (46%) and NC99-6-2 (75%) rooted best when treated with K-IBA at 15,000 mg·L^{-1}, whereas softwood cuttings of NC99-6-1 rooted best (46%) when treated with K-IBA at 10,000 mg·L^{-1}. Dwarf white and the F_1s rooted poorly in the semi-hardwood study.

Index words: adventitious rooting, auxin, K-indolebutyric acid, Fabaceae.

Species used in this study: eastern redbud (*Cercis canadensis* L.).

Significance to the Nursery Industry

Numerous clones of eastern redbud [*Cercis canadensis* L. (Fabaceae Lindl.)] are sold in the nursery trade, and they typically are propagated by budding or micropropagation. Propagation by stem cuttings, however, would provide a simpler means of propagation, although eastern redbud is regarded as difficult-to-root. In the present study, rooting response of softwood cuttings of eastern redbud was enhanced at higher concentrations of the potassium (K) salt (K-salt) of indolebutyric acid (IBA) and particular clones rooted in high percentages. The clone 'Flame' (*C. canadensis* 'Flame') was generally the strongest performer with high rooting percentages for both softwood cuttings (63%) and semi-hardwood cuttings (83%). Thus, propagation of particular clones by stem cuttings may be an alternative to more expensive methods of propagation. We suggest 'Flame' can be propagated by taking semi-hardwood cuttings during the summer and treating with K-IBA at 10,000 mg·L^{-1} (ppm).

Introduction

Eastern redbud is a small tree native to North America that is popular with landscapers because of its showy spring flowers. Several selections of eastern redbud, varying in growth habit, flower color or leaf color, are available in the nursery trade (18). Nursery professionals commonly use chip-budding or t-budding to propagate desirable clones of eastern redbud, with success rates ranging from 25 to 80% (18). Budding, however, is considered a difficult means of propagation because the bark and adhering cambium of redbud is very thin and, thus, difficult to work with (18). Suckering from the rootstock is also a common problem as the plant ages (4), and trees propagated in this manner might be prone to disease and can develop problems at the bud union (Dennis J. Werner, NC State Univ., personal communication). Because of these problems, micropropagation procedures have been developed (1, 6, 7, 8, 14, 23, 24), but concerns still remain about the vigor of redbuds produced via these methods (Pat McCracken, personal communication). It would therefore be advantageous to propagate eastern redbud by stem cuttings, but the species is generally regarded as difficult-to-root (5, 18).

Optimum rooting of eastern redbud appears to occur when cuttings are taken soon after budbreak and treated with high rates of auxin. Dillion and Klingaman (3) reported cuttings of an unidentified clone of eastern redbud prior to its release to the nursery industry rooted at 94% when taken in May and treated with IBA at 20,000 mg·L^{-1}. Stem cuttings taken in June or August rooted at 50 and 0%, respectively. Tipton (21), working with Mexican redbud [*Cercis canadensis* var. *Mexicana* (Rose) M. Hopkins], predicted maximum rooting response at 88% for cuttings taken 4 weeks after budbreak and treated with K-IBA at 21,000 mg·L^{-1}. Cuttings taken 8, 12, or 16 weeks after budbreak did not root. Tipton concluded that for optimum rooting, cuttings should be taken as soon as possible after budbreak.

Additionally, clonal variation appears to influence rooting of redbuds. For example, one clone of *C. canadensis* rooted as high at 94% (3), whereas Murphy (16) attempted to propagate 'Forest Pansy' (*C. canadensis* 'Forest Pansy') eastern redbud by hardwood cuttings and was unable to root a single cutting. Other anecdotal reports of unsuccessful

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attempts to root softwood and semi-hardwood cuttings of 'Forest Pansy' support findings of Murphy (16) and others (Dennis J. Werner, NC State Univ., personal communication; Wooldridge et al., unpublished data).

Species closely related to eastern redbud have shown greater rooting potential. Pooler and Dix (17) screened several taxa of redbud (*Cercis* L.) for rootability for inclusion in a breeding program. Semi-hardwood cuttings were taken from 2-year-old plants approximately 8 weeks after budbreak and subjected to a range of auxin treatments, including a variety of active ingredients [IBA and/or naphthaleneacetic acid (NAA)] as well as carriers (water, alcohol, or talc). Eastern redbud rooted at 56% across all auxin treatments, while Texas redbud [*C. canadensis* var. *texensis* (S. Watson) M. Hopkins] rooted at 63% across all treatments. Many Asian taxa rooted at higher levels. Chinese redbud (*C. chinensis* Bunge), smooth redbud (*C. glabra* Pampanini), Yunnan redbud (*C. yunnanensis* Hu et Cheng), and Ching's redbud (*C. chingii* Chun) rooted at 74, 100, 89, and 100%, respectively. Thomas (22) reported 75 to 90% rooting of nontreated stem cuttings of Chinese redbud. 'Softwood heel' cuttings taken in June and July rooted in 3 to 4 weeks (22). Karam and Gebre (12) investigated rooting of European redbud (Judas tree) (*Cercis siliquastrum* L.). They collected terminal and sub-terminal cuttings in April (2 to 3 weeks after budbreak), June, and January and treated the cuttings with either the free acid of IBA dissolved in 50% ethanol, K-IBA dissolved in water, or IBA as a commercial auxin-talcum powder formulation. Only cuttings taken in June rooted, and terminal cuttings performed better than sub-terminal cuttings. The highest rooting percentages (80%) were observed when cuttings were treated with K-IBA at 7200 mg·L^{-1}.

The aforementioned research suggests optimum rooting of eastern redbud most likely is achieved by taking cuttings soon after budbreak and by treating the cuttings with K-IBA at concentrations approaching 20,000 mg·L^{-1} (3, 21). These studies also indicated genotype might be a critical factor affecting rooting behavior (3, 16). Therefore, the objective of this research was to test if genotype is in fact a critical factor in identifying optimum auxin concentrations and growth stage for stem cutting propagation of eastern redbud.

Materials and Methods

Two experiments were conducted to determine the effects of auxin treatment and growth stage on rooting stem cuttings of four related clones of eastern redbud. The experiments were both 4 × 4 factorials in a randomized complete block design with four replications and six cuttings per replication. The main factors were four clones: 'Flame', dwarf white (an unreleased selection of the JC Raulston Arboretum), and two selections (NC99-6-1 and NC99-6-2) of an F_1 generation of a cross of 'Flame' and dwarf white; and four concentrations of K-IBA (0, 5000, 10,000, or 15,000 mg·L^{-1}). For each clone, cuttings were taken from a single tree in the adult growth phase. Trees of 'Flame' and dwarf white were located at the JC Raulston Arboretum, Raleigh, NC, while trees of the F_1 selections were located at the Sandhills Research Station, Jackson Springs, NC.

Softwood cuttings (Expt. 1) were taken May 10, 2006, approximately 6 weeks after budbreak. Leaves were not fully expanded, and the stems were green. When cuttings were flexed, the stems broke without a snapping sound and remained attached. Semi-hardwood cuttings (Expt. 2) of dwarf white, NC99-6-1, and NC99-6-2 were taken July 7, about 14 weeks after budbreak. At that time, the tree of 'Flame' was judged to be in an earlier growth stage than the other trees, and cuttings were not taken until 2 weeks later on July 24, about 16 weeks after budbreak. For all the semi-hardwood cuttings, the leaves were fully expanded, and stems were a medium brown color. When pressure was applied, the stem broke with a snapping sound, but did not separate.

During preparation of the cuttings at both stages of growth (Expts. 1 and 2), terminal succulent growth was removed to the first or second distal node. The cuttings were trimmed at the base resulting in a final length of 15 cm (6 in), and leaves were removed from the basal 5 cm (2 in). Leaves > 10 cm (4 in) in width were cut in half perpendicular to the midrib and the basal 2 cm (0.8 in) of each cutting was dipped for 2 sec in a solution of K-IBA at 0, 5000, 10,000, or 15,000 mg·L^{-1}. After 15 min of air drying of the IBA solutions, each cutting was set in a plastic Anderson Deep Tree Band (Anderson Tool and Die, Portland, OR) [6 × 6 × 12 cm (2.4 × 2.4 × 5 in)] containing peat:perlite (1:1 by vol). Bands (containers) were held in deep propagation flats [41 × 41 × 13 cm (16 × 16 × 5 in)] with 36 cells per flat (6 rows × 6 columns), and the flats placed under intermittent mist in a greenhouse. The mist operated from 7 am to 7 pm for 4 sec every 10 min in the softwood study and 4 sec every 8 min in the semi-hardwood study. Evaporative cooling was activated in the greenhouse if the temperature rose above 21C (70F). Heating was activated if the greenhouse temperature dropped below 16C (60F). Cuttings were left in the mist for 8 weeks.

Experiment 1 was terminated July 5. In Expt. 2, cuttings of dwarf white, NC99-6-1, and NC99-6-2 were removed and data recorded September 18; cuttings of 'Flame' were removed and data recorded October 2. A cutting having one primary root ≥ 1 mm (0.04 in) in length was classified as rooted. After recording the number of primary roots, the length of each primary root was measured for the softwood study. In Expt. 2, the number of roots was recorded and total root length and total root area were obtained using a Monochrome Agvision System 286 Image Analyzer (Decagon Devices, Inc., Pullman, WA). For both studies, the roots were dried at 65C (149F) for at least 48 hr and then weighed. Rooting percentages were calculated, and data were subjected to analysis of variance (ANOVA) and regression analysis, where appropriate. When regression analysis was significant, linear and quadratic curves were fitted to data. The maximum of the quadratic curve was calculated as a first order derivative of the independent variable where the dependent variable equaled zero.

Results and Discussion

Softwood cuttings (Expt. 1). Rooting percentage, root number, total root length, and total root dry weight were significantly affected by clone and K-IBA treatment, whereas the clone by K-IBA rate interaction was not significant (Table 1). K-IBA had a linear effect on rooting percentage (Table 2). Averaging across all clones, nontreated cuttings rooted at a mean of 10% whereas cuttings treated with K-IBA at 15,000 mg·L^{-1} rooted at 55%. For those cuttings which rooted, K-IBA had a linear effect on root number, and total root length, while K-IBA had a quadratic effect on total root dry weight (Table 2).

Averaged across clones and K-IBA rate, total rooting of the softwood cuttings was 38% when taken 6 weeks after

Table 1. ANOVAs for rooting percentage, root number, total root length, and total root dry weight of softwood cuttings of eastern redbud as influenced by clone, K-IBA rate, and clone by K-IBA rate interaction (Expt. 1).

Treatment	Rooting (%)	Root no.[z]	Total root length[z]	Total root dry wt.[z]
Clone	0.0101	0.0002	0.0234	0.0062
K-IBA rate	< 0.0001	0.0161	0.0056	0.0051
Clone × K-IBA rate	0.3629	0.3393	0.3631	0.2513

[z]Rooted cuttings only.

Table 2. Rooting response of softwood cuttings of four clones ('Flame', NC99-6-1, NC99-6-2, and dwarf white) of eastern redbud as influenced by K-IBA treatment (Expt. 1).

K-IBA concn. (mg·L^{-1})	Rooting[z] (%)	Root no.[y]	Total root length[y] (cm)	Total root dry wt.[y] (mg)
0	10 ± 4	2.6 ± 1.1	6.2 ± 3.3	6.5 ± 2.8
5000	42 ± 7	4.3 ± 0.7	20.4 ± 3.7	19.6 ± 4.1
10,000	44 ± 5	6.8 ± 1.5	33.0 ± 6.6	27.2 ± 4.6
15,000	55 ± 6	6.1 ± 0.9	25.0 ± 3.3	19.7 ± 2.2
Linear[x]	***	*	*	NS
Quadratic	NS	NS	*	*

[z]Data are means based on four replications per clone with six cuttings per replication ± 1 SE.

[y]Data are means based on four replications and the number of cuttings which rooted per replication ± 1 SE.

[x]NS, *, *** Nonsignificant or significant at $P < 0.05$, or 0.001, respectively.

Regression equations are: rooting(%) = 3.6 + 1.4x, R^2 = 0.84; root no. = 1.7 + 1.3x, R^2 = 0.78; total root length (linear) = 3.9 + 6.9x, R^2 = 0.63; total root length (quadratic) = –23.7 + 34.5x – 5.5x^2, R^2 = 0.95; total root dry wt. = –19.3 + 30.5x – 5.1x^2, R^2 = 0.98.

Table 3. Rooting response of softwood cuttings of four clones of eastern redbud averaged over four rates of K-IBA (Expt. 1).

Clone	Rooting[z] (%)	Root no.[y]	Total root length[y] (cm)	Total root dry wt.[y] (mg)
'Flame'	50 ± 6a[x]	8.5 ± 1.5a	30.6 ± 7.2a	25.8 ± 5.2a
NC99-6-2	42 ± 8ab	5.1 ± 0.7b	28.4 ± 3.8a	26.0 ± 3.7a
NC99-6-1	31 ± 6b	4.3 ± 0.8b	18.3 ± 3.7ab	12.8 ± 2.5b
dwarf white	28 ± 6b	2.6 ± 0.3b	14.8 ± 2.7b	15.4 ± 2.7b

[z]Data are means based on four replications per clone with six cuttings per replication ± 1 SE.

[y]Data are means based on four replications and the number of cuttings which rooted per replication ± 1 SE.

[x]Mean separation within columns by Fisher's LSD at $P < 0.05$.

budbreak. Pooler and Dix (17) reported 56 and 63% rooting for semi-hardwood cuttings of eastern redbud and Texas redbud, respectively. Those cuttings, however, were taken from plants in the juvenile growth phase, and cuttings from juvenile plants typically produce adventitious roots more readily than cuttings taken from plants in the adult growth phase (10). Comparison of our results to those of Pooler and Dix (17) is further confounded because the rooting percentages presented by Pooler and Dix (17) were the average of four different auxin treatments, including a variety of active ingredients (IBA and/or NAA) as well as carriers (water, alcohol, or talc). In addition, there was no nontreated control. Tipton (21), working with softwood cuttings of Mexican redbud, predicted rooting of 88% when cuttings were taken 4 weeks after budbreak. Mexican redbud, however, has a reputation for rooting in higher percentages than other species of the genus (Pat McCracken and Dennis J. Werner, personal communication). Dillion and Klingaman (3) reported cuttings taken in May rooted at 94%, although there is no mention of how many weeks after budbreak the cuttings were taken.

As noted above, clone had a significant affect on rooting. For every dependent variable measured, 'Flame' performed at a higher level than dwarf white. The F_1s generally performed intermediate to 'Flame' and dwarf white (Table 3). This suggests a genetic influence with additive gene action on the rootability of eastern redbud, though additional research would be necessary to confirm this hypothesis.

Reports of clonal differences in the rooting of stem cuttings are not uncommon for both angiosperms and gymnosperms (9). Zimmerman and Hitchcock (25) reported as early as 1929 on the effect of genotype on the rooting of American holly (*Ilex opaca* L.). More recently, the effect of genotype on the rooting capacity of stem cuttings has been reported for many species including Fraser fir [*Abies fraseri* (Pursh) Poir.] (15), eastern redcedar (*Juniperus virginiana* L.) (11), common cypress (*Cupressus sempervirens* L.) (2), lemon myrtle (*Backhousia citriodora* F. Muell) (13), sycamore (*Platanus occidentalis* L.) (19), and hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) (20).

Overall, 'Flame' rooted most successfully, but the highest single rooting percentage was for cuttings of NC99-6-2 treated with K-IBA at 15,000 mg·L^{-1}. Those cuttings rooted at 75% with an average of 6.1 roots per cutting, a total root length of 30.6 cm (11.9 in) and total root dry weight of 25 mg (0.0009 oz) (data not presented). 'Flame' rooted in the highest percentage (63%) when treated with K-IBA at 5000 mg·L^{-1}. However, cuttings of 'Flame' treated with K-IBA at 10,000 mg·L^{-1} had larger root systems [13.1 roots, total root length of 57.3 cm (22.3 in), and total root dry weight of 46 mg (0.0016 oz)], the largest of any treatment-clone combination. Cuttings of NC99-6-1 and dwarf white rooted best (both 46%) when treated with K-IBA at 10,000 and 15,000 mg·L^{-1}, respectively. For these treatments, rooted cuttings of NC99-6-1 and dwarf white had an average number of roots, total root length, and total root dry weight of 6.9 and 3.8 roots, 29.1 cm (11.3 in) and 17.1 cm (6.7 in), and 17 mg (0.0006 oz) and 15 mg (0.0005 oz), respectively.

Semi-hardwood cuttings (Expt. 2). For semi-hardwood cuttings taken in July, rooting percentage was again affected by clone and K-IBA treatment. In addition, the clone by K-IBA interaction was significant for rooting percentage (P = 0.0001) (Table 4).

'Flame' rooted well, while dwarf white, NC99-6-1, and NC99-6-2 rooted poorly (Fig. 1). Dwarf white did not root regardless of K-IBA treatment. At each concentration of K-IBA other than 0 mg·L^{-1}, 'Flame' rooted at a significantly higher percentage than the other clones. Treating cuttings of dwarf white, NC99-6-1, and NC99-6-2 with K-IBA did not

Table 4. ANOVAs for rooting percentage, root number, total root length, total root area, and total root dry weight of semi-hardwood cuttings of eastern redbud as influenced by clone, K-IBA rate, and clone by K-IBA rate interaction (Expt. 2).

Treatment	Rooting (%)	Root no.[z]	Total root length[z]	Total root area[z]	Total root dry wt.[z]
Clone	< 0.0001	< 0.0001	0.0061	0.0005	< 0.0001
K-IBA rate	< 0.0001	0.1762	0.1972	0.1355	0.2985
Clone × K-IBA rate	0.0001	0.0487	0.0866	0.1118	0.1604

[z]Rooted cuttings only.

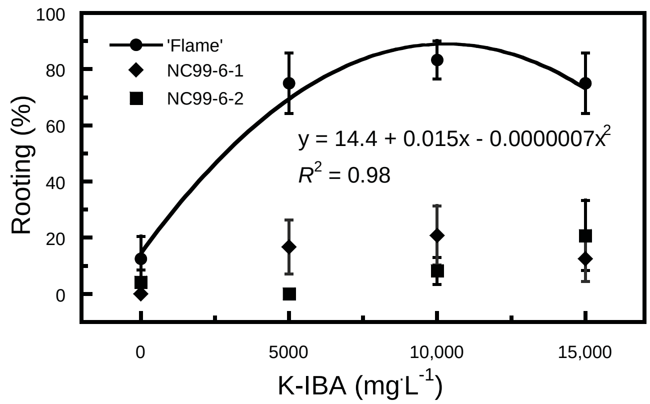

Fig. 1. Rooting response of semi-hardwood cuttings of three clones of eastern redbud as influenced by K-IBA treatment (Expt. 2). Data points are means based on four replications per clone with six cuttings per replication. Vertical bars = ± 1 SE.

stimulate rooting, as all measured variables were not affected by rate of K-IBA (data not presented).

Stem cuttings of 'Flame' treated with K-IBA at 10,000 $mg \cdot L^{-1}$ rooted at 83% (Fig. 1). These cuttings had a mean of 15.3 roots, mean total root length of 80.9 cm (31.6 in), mean total root area of 10.4 cm^2 (1.6 in^2), and total root dry weight of 77 mg (0.0027 oz) (Table 5). The rooting response of semi-hardwood cuttings of 'Flame' to K-IBA treatment was quadratic ($R^2 = 0.98$) with maximum rooting percentage predicted at 10,700 $mg \cdot L^{-1}$. There was also a quadratic response of root number, total root length, total root area, and total root dry weight to increasing concentrations of K-IBA (Table 5).

While semi-hardwood cuttings of dwarf white, NC99-6-1, and NC99-6-2 rooted poorly compared to softwood cuttings, semi-hardwood cuttings of 'Flame' rooted in higher percentages and had larger, more robust root systems than softwood cuttings of 'Flame'. These results were surprising as Tipton (21) reported stem cuttings of Mexican redbud did not root when taken 8, 12, or 16 weeks after budbreak. Similarly, Dillion and Klingaman (3) observed that cuttings of an unnamed clone taken after June did not root. In contrast, cuttings of 'Flame' taken in the present investigation on July 24, 16 weeks after budbreak, rooted in percentages as high as 83%.

The rooting response of softwood or semi-hardwood cuttings differed among clones of eastern redbud. In the softwood study, 'Flame' outperformed dwarf white for each response measured; in the semi-hardwood study, 'Flame' rooted better than the other clones for each level of K-IBA > 0 $mg \cdot L^{-1}$.

Although the present studies were not designed to compare statistically the influence of growth stage on rooting, dwarf white, NC99-6-1, and NC99-6-2 rooted in higher percentages in the softwood study, whereas 'Flame' had a higher rooting percentage in the semi-hardwood study. This was surprising as previous research indicates optimum rooting of stem cuttings of eastern redbud is achieved when cuttings are taken soon after budbreak (3, 21).

Results herein suggest propagation protocols for eastern redbud are clonally dependent. The clones investigated did not respond similarly, nor did 'Flame' respond in a manner consistent with reports of similar clones and species (3, 21). It is unlikely a single protocol for propagation by stem cuttings would yield satisfactory results for all selections of eastern redbud. However, propagation by stem cuttings may be commercially feasible for particular genotypes of eastern redbud as evidenced by the strong rooting response of 'Flame.' In the softwood study, 'Flame' rooted at 63% and had well developed root systems. In the semi-hardwood study 'Flame' rooted at 83%, with even more robust root systems. It seems likely 'Flame' can be rooted in commercially acceptable percentages with cuttings taken in May through July and treated with K-IBA at 10,000 $mg \cdot L^{-1}$.

Table 5. Rooting response of semi-hardwood cuttings of 'Flame' eastern redbud as influenced by K-IBA treatment (Expt. 2).[z]

K-IBA concn. ($mg \cdot L^{-1}$)	Root no.	Total root length (cm)	Total root area (cm^2)	Total root dry wt. (mg)
0	5.8 ± 2.8	23.9 ± 10.1	3.0 ± 1.2	28 ± 13
5000	11.6 ± 2.4	63.1 ± 6.0	8.0 ± 1.0	61 ± 11
10,000	15.3 ± 2.0	80.9 ± 4.7	10.4 ± 0.8	77 ± 4
15,000	11.6 ± 0.9	55.5 ± 8.7	8.1 ± 1.0	58 ± 8
Linear[y]	NS	NS	NS	NS
Quadratic	*	***	**	*

[z]Data are means based on four replications and the number of cuttings which rooted per replication ± 1 SE.

[y]NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Regression equations are: root no. = $5.49 + 0.0018x - 0.000000096x^2$, $R^2 = 0.97$; total root length = $22.83 + 0.012x - 0.00000065x^2$, $R^2 = 0.99$; total root area = $2.87 + 0.0015x - 0.000000074x^2$, $R^2 = 0.99$; total root dry wt. = $27.14 + 0.010x - 0.00000052x^2$, $R^2 = 0.99$.

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