Micropropagation of *Acer platanoides* L. 'Crimson Sentry'

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Significance to Industry: Norway maple (*Acer platanoides* L.) is a valuable landscape tree known for its attractive foliage and architecture. Since its introduction to North America by Bartram in 1756 (10), Norway maple has played a major role in replacing the American elm following Dutch elm disease (13, 14). Pest and disease resistance, and tolerance of poor soils have made Norway Maple a popular choice as a municipal street tree. However, Norway maple has become invasive in disturbed forests, along roadside edges, and within intact forests bordering ornamental plantings in the Northeastern and Midwestern United States (3, 12, 13). Inducing autopolyploids may improve ornamental features of Norway maple and provide an opportunity to develop triploid, seedless cultivars. In this study, in vitro regeneration protocols were developed for micropropagation and as a platform for future ploidy manipulation.

Nature of Work: In vitro regeneration provides an excellent method for rapid propagation. In vitro shoot regeneration studies for *A. platanoides* have primarily focused on wild types or the popular cultivar 'Crimson King' (4, 7, 8). These studies investigated different combinations of basal salts such as Murashige and Skoog (MS) (9), Linsmaier and Skoog (LS) (5), and Lloyd and McCown (WPM) (6) and cytokinins such as zeatin, kinetin (Kin), thidiazuron (TDZ) and 6-benzylaminopurine (BAP) to maximize in vitro shoot regeneration (1, 2, 4, 7, 8). Further in vitro rooting studies with *A. platanoides* have investigated low salt media supplemented with the synthetic auxin 3-indolebutyric acid (IBA) (1, 8). Thus, the objectives of this research were to develop in vitro multiplication and rooting protocols for *A. platanoides* 'Crimson Sentry'.
Stock explants for all experiments were maintained in vitro on *Acer* medium containing MS basal salts and vitamins, 2 µM BAP, 100 $mg \cdot L^{-1}$ myo-inositol, 100 $mg \cdot L^{-1}$ MES buffer, 30 $g \cdot L^{-1}$ sucrose, pH 5.75, and solidified with 7.5 $g \cdot L^{-1}$ agar in 180 cc jars. All samples were incubated at 23°C under a 16-h photoperiod (cool white 40W fluorescent lamps with PPFD 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

The effect of MS, WPM, and Quoirin and Lepoivre (QL) (11) basal salts in factorial combination with the cytokinins BAP, TDZ, 2-isopentenyladenine (2iP), meta-topolin (*m*T), and Kin on in vitro growth of 'Crimson Sentry' was examined. All media treatments were supplemented with 100 $mg \cdot L^{-1}$ myo-inositol, 100 $mg \cdot L^{-1}$ MES buffer, 30 $g \cdot L^{-1}$

sucrose, 7.5 $g \cdot L^{-1}$ agar, and 2 µM of cytokinin with media pH adjusted to 5.75. Six replicates, each with 5 shoots (subsamples), were incubated on each media composition under standard culture conditions using a completely randomized design. After 5 weeks, data were collected on the number of shoots, shoot length (of the longest shoot), number of leaves, and number of nodes (on the longest shoot). Data sets were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (version 9.1; SAS Institute Inc, Cary, NC) (Table 1).

In a separate experiment, the optimum concentration of BAP for shoot multiplication was evaluated. Media containing MS basal salts and vitamins, 100 $mg \cdot L^{-1}$ myo-inositol, 100 $mg \cdot L^{-1}$ MES buffer, 30 $g \cdot L^{-1}$ sucrose, and 7.5 $g \cdot L^{-1}$ agar with a of pH 5.75 was supplemented with BAP at 0, 2, 4, 8 or 16 µM. Seven replicates per treatment with 5 subsamples (2- to 3-cm shoots) were arranged in a completely randomized design under standard culture conditions. After 5 weeks, data were collected on the number of shoots, shoot length (of the longest shoot), number of leaves, and number of nodes (on the longest shoot). Data were subjected to trend analysis using the GLM procedure of SAS (Table 2).

For studies on in vitro rooting, media consisted of half-strength WPM basal salts and vitamins and 30 $g \cdot L^{-1}$ sucrose, supplemented with 0, 5, 10, 20, 40 or 80 µM IBA. All media were adjusted to a pH of 5.75. Each treatment consisted of 6 replications. Five subsamples (1- to 2-cm shoots) were assigned to each treatment, and all were arranged in a complete randomized design under standard culture conditions. Following 8 weeks of growth, data were collected on rooting percentage and root length (longest root) (Table 3). Data were subjected to trend analyses using SAS (Table 3).

Results and Discussion: Shoot regeneration was achieved with all treatments of basal salts and cytokinins. ANOVA results showed that basal salts, cytokinins, and their interactions significantly affected both shoot length ($P \leq 0.01$) and number of nodes ($P \leq 0.05$) (Table 1). The combination of MS and 2 µM BAP produced the longest mean shoot length (30.6+1.4 mm), and there was a general trend among all media types of BAP producing longer shoots. The combination of MS + BAP and QL + BAP also produced the most nodes (3.6+0.2 and 3.5+0.2, respectively). Shoot number was influenced only by cytokinin type ($P \leq 0.01$). In general, BAP and *m*T produced the highest number of shoots across all basal salt treatments. Both medium and cytokinin type significantly influenced mean leaf number ($P \leq 0.01$ and 0.01, respectively) but not their interaction.

In the second experiment, trend analysis of shoot data revealed that shoot length has a negative linear response to BAP concentration ($P \leq 0.01$) (Table 2). There was no significant effect of BAP concentration on shoot number. However, multiplication rate and number of 20 mm explants obtained per replicate per 6 weeks exhibited a quadratic response to BAP ($P \leq 0.01$) with the best BAP concentration estimated at 6 µM.

For the rooting experiment, in vitro root formation was observed in all treatments after 8 weeks. Trend analysis showed percent rooting, number of roots per rooted cuttings, and root length of microcuttings of *A. platanoides* 'Crimson Sentry' all exhibited a quadratic response to IBA concentration ($P \leq 0.01$, 0.01, and 0.05, respectively) (Table 3). Based on trend analysis, the highest percent rooting and longest roots was achieved using between 5 and 10 µM IBA. The highest number of roots produced per microcutting was achieved using between 5 and 40 µM IBA.

This study demonstrated that there are several different media components that interact to influence in vitro growth of *A. platanoides* 'Crimson Sentry'. Based on the results, MS media supplemented with 6 µM BAP provided high shoot proliferation, while half-strength WPM supplemented with 10 µM IBA produced the best rooting results. Protocols developed in this study will be used in future experiments focused on the in vitro development of autopolyploids.

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Table 1. Effect of media composition and cytokinin on in vitro growth of *Acer platanoides* 'Crimson Sentry'.

Media	Cytokinin	Shoots (no.)[z]	Shoot length (mm)[z]	Leaves (no.)[z]	Nodes (no.)[z]
MS	BAP	3.2 ± 0.3ab	30.6 ± 1.4a	8.1 ± 1.0ab	3.6 ± 0.2a
	TDZ	0.9 ± 0.04c	10.8 ± 0.5g	2.4 ± 0.4g	1.2 ± 0.1h
	2iP	1.9 ± 0.7d	12.0 ± 0.9fg	6.3 ± 1.1cde	2.2 ± 0.2ce
	*m*T	3.1 ± 0.2ae	21.4 ± 2.4bc	7.5 ± 0.6ac	2.9 ± 0.2b
	Kin	1.8 ±0.2d	9.9 ± 1.1g	5.5 ± 0.6de	1.9 ± 0.2ef
WPM	BAP	3.9 ± 0.5ab	22.1 ± 1.8bc	7.8 ± 0.8ac	2.9 ± 0.2b
	TDZ	0.9 ± 0.03c	12.0 ± 0.9f	2.4 ± 0.4g	1.4 ± 0.1gi
	2iP	2.1 ± 0.3df	15.0 ± 0.8ef	4.9 ± 0.2ef	2.1 ± 0.1de
	*m*T	2.9 ± 0.4af	19.7 ± 3.1cd	6.8 ± 0.5bcd	2.6 ± 0.2bc
	Kin	2.3 ± 0.1def	10.9 ± 0.5g	4.5 ±0.2ef	1.8 ±0.1e
QL	BAP	3.5 ± 0.2AB	24.8 ± 3.0b	8.9 ± 0.5a	3.5 ± 0.2a
	TDZ	0.9 ± 0.1c	10.3 ± 1.6g	2.2 ± 0.4g	1.1 ± 0.2i
	2iP	1.7 ± 0.3cd	9.2 ± 0.2g	3.7 ± 0.5fg	1.6 ± 0.1fgh
	*m*T	2.5 ± 0.3def	16.9 ± 1.5de	5.9 ± 0.6de	2.4 ± 0.2cd
	Kin	2.4 ± 0.2def	10.1 ± 0.4g	3.6 ± 0.3fg	1.8 ± 0.2eg
Analysis of Variance:					
Media		NS[y]	*	**	*
Cytokinin		**	**	**	**
Media x Cytokinin		NS	**	NS	*

[z]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P ≤ 0.05.
[y]NS, *, **: Nonsignificant or significant at P ≤ 0.05 and 0.01, respectively.

Table 2. Influence of BAP[z] concentration on in vitro shoot growth of *Acer platanoides* 'Crimson' Sentry'.

BAP (µM)	Shoots (no.)[y]	Shoot length (mm)[y]	Multiplication rate (explants per jar after 6 weeks)[y]
0	1.4 ± 0.2	17.4 ± 0.4	7.0 ± 0.8
2	2.3 ± 0.4	27.4 ± 2.1	14.2 ± 0.3
4	2.5 ± 0.2	25.5 ± 1.7	14.2 ± 1.7
8	2.0 ± 0.1	13.3 ± 1.0	9.2 ± 0.8
16	2.4 ± 0.2	12.9 ± 0.8	6.0 ± 0.4
Trend analysis:			
Linear	NS[x]	**	*
Quadratic	NS	NS	**

[z]BAP = 6-benzylaminopurine.
[y]Values represent means ± SE.
[x]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively.

Table 3. Influence of IBA[z] concentration on in vitro rooting of *Acer platanoides* 'Crimson Sentry'.

IBA (µM)	Rooting (%)[y]	Roots (no.)[y]	Root length (mm)[y]
0	0 ± 0	0.0 ± 0.0	0.0 ± 0.0
5	77 ± 13	2.6 ± 0.8	17.1± 3.1
10	70 ± 12	3.0 ± 0.9	17.9 ± 3.5
20	53 ± 13	1.8 ± 0.7	11.9 ± 2.7
40	63 ± 10	3.4 ± 1.0	11.5 ± 1.4
80	13 ± 7	0.2 ± 0.1	3.7 ± 1.7
Trend analysis:			
Linear	NS	NS	NS
Quadratic	**	**	*

[z]IBA = indolebutyric acid.
[y]Values represent means ± SE.
[x]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively.