Rooting Efficiency of Amur Maple Seedless Selections Produced by Mutagenesis

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Abstract

Amur maple (Acer ginnala Maxim.) is a widely planted small tree with attractive red fall foliage. In many states, it is classified as a noxious weed and regulations restrict propagation and sale, limiting customer choice. Stem cuttings are commonly used as a means of asexual propagation of Amur maple, with softwood cuttings being the easiest to root. Here we report adventitious rooting efficiency of seedless Amur maple selections using four indole-3-butyric acid (IBA; 500; 1,500, 5,000, 10,000 ppm) and four naphthalene acetic acid (NAA; 100, 500, 1,500, 5,000 ppm) treatments and a no hormone control. Overall, cuttings treated with 5,000 or 10,000 ppm IBA and 5,000 ppm NAA resulted in the highest percent rooting (PR), mean number of roots (MNR), and mean length of the longest root (MRL) across all seedless selections. Seedless selections SW-30-14 and SW-30-130 had the highest PR, 66% and 67%, respectively, and will perform well in commercial propagation. This level of rooting was greater than that of the cultivar ‘Bailey Compact’ that had 46% PR, and the seedless selection SW-30-159 that had the lowest PR (10.3%), MNR (0.3), and MRL (0.2 in) averaged across all treatments. Cuttings from 4 of the 5 seedless selections treated with 1,500 ppm NAA had the greatest PR, whereas ‘Bailey Compact’ and SW-30-159 had the greatest PR with 5,000 ppm NAA. Results indicate that rooting efficiency was impacted by mutagenesis in some selections, which may require optimization of propagation methods for those selections.

Index words: adventitious rooting, auxin, vegetative propagation, sterile, non-native invasive.

Species used in this study: Amur maple (Acer ginnala Maxim.).

Chemicals used in this study: indole-3-butyric acid (IBA; 500, 1,500, 5,000, 10,000 ppm) and naphthalene acetic acid (NAA; 100, 500, 1,500, 5,000 ppm).

Significance to the Horticulture Industry

This is an ongoing project researching mutagenesis breeding of Amur maple to reduce seed production and invasiveness. Amur maple is classified as a noxious or invasive plant and regulations restrict propagation and sale of this plant limiting customer choice. The seedless selections were used to conduct this rooting experiment. Commercialization of seedless Amur maple selections requires the development of a suitable asexual propagation method; therefore, a rooting experiment was performed. Seedless selections of Amur maple produced through mutagenesis respond differently to growth hormone treatments and levels and the majority of the selections can be efficiently propagated.

Introduction

Non-native plants are a source of new horticultural introductions that bring novelty and become popular with consumers and the nursery industry. Unfortunately, a small number of non-native plant introductions escape cultivation, establish in new environments and become invasive (Lockwood et al. 2001, Lockwood et al. 2005). The cost associated with eradication of invasive species in the United States are estimated to be $137 billion per year (Pimentel et al. 2000). Amur maple (or Ginnala maple) is an example of this type of non-native invasive introduction to North America. However, Amur maple is a recognized landscape plant with attractive red fall foliage and pink fruit. Due to its outstanding ornamental value, Amur maple is extensively planted as shelterbelts, hedges, and wind screens, as well as in urban gardens or as a residential street tree. In addition to its aesthetic value, Amur maple is easy to propagate and is low maintenance, making it popular for consumers and growers. Amur maple thrives in a wide range of environments from USDA hardiness zones 3 through 8 (Gilman and Watson et al. 1993).

The key characteristics associated with invasive plants are: high seed production, efficient seed dispersal, rapid growth, tolerance to a wide range of environments, and lack of pests and diseases (Mack et al. 2000, Correia et al. 2016, Herrera et al. 2011). In the Northern United States, Amur maple is a specially regulated plant. Amur maple has prolific seed production and can displace native plants, causing economic and environmental damage. In Minnesota, it must be labeled with the warning to only be planted at least 100 yards from natural areas (Minnesota Department of Natural Resources – Amur maple, 2019).

As a response to Amur maple’s invasive potential and to maintain its high horticultural value, a mutagenesis breeding project was initiated at the University of Minnesota with a goal of producing seedless Amur maple selections (Smith and Noyszewski 2018). Prolific seed set is a major determinant of invasiveness, so elimination or reduction of seeds can eliminate invasive potential (Leishman et al. 2000). In addition, seedlessness may increase...
vegetative growth, further improving a new variety. Multiple methods exist to produce seedlessness, such as producing triploid plants by crossing a tetraploid with a diploid plant, regenerating plants from the triploid endosperm, mutagenesis, and genetic engineering or traditional breeding that produces genotypes with a reduced number of seeds (Smith et al. 2006). Mutagenesis breeding has been used to produce genetic variation, including seedlessness, in many plant species (Osborne and Lunden 1961, FAO/IAEA Mutant Variety Database). The genetic variation may be single nucleotide changes, major chromosomal rearrangements or DNA insertions and deletions.

Adventitious rooting of softwood cuttings is the primary method of maple propagation and is broadly used in the nursery industry (Chapman et al. 1979, Maynard and Bassuk 1990, Wells et al. 1999). Rooting is influenced by many factors including genotype, time of year when cuttings are collected, auxin type and concentration, auxin solvent, temperature, light intensity, and misting (Osterc and Štampar 2011, Gabriel et al. 1961, Toussignant et al. 2003, Preece et al. 2002). In combination, these factors play a pivotal role in adventitious rooting efficiency and establishment of healthy trees. The two auxins commonly used for stimulating rooting in woody plants are indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) (Dirr and Heuser 1987, Dirr 1986), with IBA being used most frequently. A majority of the rooting studies focused on rooting of Japanese maple (A. palmatum Thunb.) or other maples, but not Amur maple. A common hormone treatment for Japanese maple is 2,000 ppm of IBA as a spray mixture. Dirr 1990 used 500 ppm of IBA and P-ITB (phenyl indole-3-thiobutyrate) and 2,000 ppm P-ITB on Amur maple ‘Compactum’. Treatments of IBA and P-ITB at 500 ppm produced similar levels of adventitious rooting.

The 2,000 ppm P-ITB treatment was used to evaluate cytotoxicity of P-ITB, but no cytotoxicity was observed, and the treatment did not increase rooting when compared to the 500 ppm P-ITB treatment. In addition, a range of IBA concentrations (0, 500 and 1,500 ppm) were tested, but the effects on PR and root count were not significantly different (Dirr 1990).

Five Amur maple mutagenized selections did not flower or set fruit for 10 years, while the majority of the same-aged population (control and mutagenized) flowered and set prolific seed (Smith and Noyszewski 2018). It appears that the Amur maple selections have a defect that results in lack of flowering and therefore no fruit production, resulting in seedlessness (Smith and Noyszewski 2018). Five seedless selections and the cultivar ‘Bailey Compact’ were used to conduct this rooting experiment. Commercialization of seedless Amur maple selections requires the development of a suitable asexual propagation method; therefore, an experiment measuring adventitious rooting of cuttings was performed.

The objective of this research was to test auxin types and concentrations for the propagation of softwood stems cuttings of seedless Amur maple selections to determine whether the mutagenesis treatments affected competency for adventitious rooting. We used three measures of rooting efficiency: 1) percent of rooting, 2) mean number of roots and 3) mean length of the longest root.

Material and Methods

Amur maple selections used and statistical analysis. Field grown Amur maple plants were monitored and five mutagenized individuals (12-years-post irradiation as of 09/19) were identified as nonflowering, seedless or with reduced seed production (Smith and Noyszewski 2018). The selected seedless plants do not flower or flowered at a greatly reduced rates (Smith and Noyszewski 2018). Only a single plant was available for each seedless selection that included SW-30-14, SW-30-70, SW-30-130, SW-30-159, and SW-30-161. These individuals and Amur maple ‘Bailey Compact’ (control) were used for a preliminary rooting experiment performed in 2016. Softwood cuttings were collected on 14 June, 2016, treated with NAA and IBA and evaluated. In 2017, a larger experiment used seven replicate cuttings placed randomly in one of 9 hormone treatments and was replicated once in time (cuttings collect on 1 and 7 June, 2017), providing 14 cuttings per treatment for evaluation (Taylor et al. 2018, Fersus et al. 2017). In total 882 cuttings were evaluated. Terminal softwood cuttings were collected from each field-grown selection in Cottage Grove, Minnesota. Statistical analyses were performed with IBM SPSS v. 25 (IBM Corp. Released 2017, IBM SPSS Statistics for Windows, Version 25.0. IBM Corp., Armonk, NY). Differences between treatments, auxin type, concentrations and selections were tested by Tukey’s honest significance test at α = 0.05.

Rooting medium preparation. Sureroots® 50 plug tray (PL-SR-50-DP-VH, T.O. Plastics, Clearwater, MN) were disinfected with Roccald-D (Pfizer, New York, NJ) and placed in flats without drainage (SL1400235; SunBlast® Holdings ULC, Langley, BC, Canada). Steam sterilized (1h) sand was used as the rooting medium. Each plug tray was filled to within 2.5 cm (1 in) of the top of each pack with sand (Fig. 1) and treated with 0.48 mg L⁻¹ (0.062 oz gal⁻¹) of Banrot (The Scotts Company, Marysville, OH) as a drench.

Cuttings preparation. Cuttings collected from the field were approximately 20 cm in length (Fig. 2A) and kept wet in polyethylene bags at 4 C for 4-5 hours before hormone treatment and inserting into the rooting medium. Cuttings were trimmed to approximately 15 cm (6 in) in length from the distal (youngest) end while maintaining 4 leaves (Fig. 2B). After trimming, cuttings were disinfected with a 10% bleach solution for 10 min and rinsed with tap water three times. Excess water was removed by blotting on paper towels. Disinfected cuttings were placed in clean polyethylene bags.

Growth hormone treatment. Based on the results of our preliminary experiments in 2016 (data not shown), shoots were treated with 500, 1,500, 5,000, 10,000 ppm IBA (Hortus IBA, Earth City, MO) or 100, 500, 1,500, 5,000 ppm NAA (PhytoTech LABS, Lenexa, KS) or a water control for a total of nine treatments. The IBA and NAA were solubilized in water and 95% ethanol, respectively.
The proximal end of each cutting [2.5 cm (1 in)] was dipped for 30 seconds in each hormone solution and the cuttings placed to a depth of 5 cm into the rooting medium (Fig. 1). Plug trays with cuttings were placed in a greenhouse mist room with 8 seconds of misting every 8 minutes until cuttings showed formation of adventitious roots (at 9 weeks) that would sustain growth of cuttings without misting, at which time rooting was evaluated.

**Rooting evaluation.** Three measures of rooting efficiency were made: 1) percent of rooting (measured as the number of shoots that produced any roots/the total number of cuttings x 100%), 2) mean number of roots (measured as a total number of roots/the total number of cuttings), 3) mean length of the longest root (the longest root of each cutting within a treatment was measured and a mean calculated). A cutting was considered rooted if it had one or more roots of length equal to or greater than 0.1 in (0.25 cm) in length. Each measurement was performed for each cutting within each treatment at 9 weeks after placing the cuttings in rooting medium.

**Results and Discussion**

**Hormone type and concentrations.** Overall, cuttings treated with IBA and NAA had higher PR, MNR and MRL relative to water treatments (control) across all selections (Fig. 3A1, A2, and A3). Cuttings treated with low levels of NAA (100 ppm) and IBA (500 ppm) had PR, MNR, and MLL that were not significantly different from cuttings treated only with water (control). The highest levels of auxin (1,500 and 5,000 ppm NAA; 5,000 and 10,000 ppm IBA) had a similar PR. The numerically highest PR (71%) was achieved with 1,500 ppm NAA; however, 5,000 ppm NAA, 5,000 IBA and 10,000 ppm IBA treatments produced statistically similar levels of PR, MNR and MRL (Fig. 3B1). These data show a significant effect of hormone treatment and the selection, but there was no significant interaction between the two factors. Comparable results were reported by Chapman et al. (1979) and Dirr et al. (1990) with 86% rooting using 8,000 IBA and 67% rooting using 5,000 ppm, P-IBA, although Enright et al. (1958) achieved 100% PR rooting using 20,000 ppm, IBA. Cuttings treated with 500, 1,500 and 5,000 ppm NAA and 5,000 and 10,000 ppm IBA treatments have greater...
MNR when compared to control (water) or lowest auxin levels (Fig. 3B2). Cuttings with the highest MNR levels had a mean of 3 to 4 roots per cutting, which should be sufficient for continued growth. Low MNR was observed for cuttings treated with water, 100 ppm NAA, 500 IBA and 1,500 ppm IBA treatments. These data were consistent with those of Dirr et al. 1990 that reported 4 to 9 roots per cutting (depend on the treatment). The numerically longest MRL (approximately 2 in) occurred at 1,500 ppm NAA treatment, with 5,000 ppm NAA and 10,000 ppm IBA producing similar results (Fig. 3B3). These data suggest standard rooting methods will work with seedless selections, but more optimization (timing of cutting harvest, auxin application methods, rooting medium, temperature and heating methods while rooting and manipulation of lighting) may be needed to achieve higher levels of PR.

Fig. 3. Overall main effect of hormone type, level and genotype on Amur maple rooting A) percentage (PR), B) mean number of roots/cutting (MNR) and C. mean root length (MRL). Mean separations are indicated by a - f as determined by Tukey’s test (p = 0.05) and error bars are standard error. Abbreviations: indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), parts per million (ppm).
Fig. 4. Effect of auxin type and level on A. percent rooting (PR), B. mean number of roots per cutting (MNR) and C. mean root length (MRL). Mean separations are indicated by a - d as determined by Tukey’s test (p = 0.05), error bars as standard error. Abbreviations: indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), parts per million (ppm).
Amur maple selection responses. When treatment data where combined and evaluated for each selection, the PR, MNR and MRL differed (Fig. 3. C1, C2 and C3; Selections). The seedless selection SW-30-159 had the lowest PR (10.3%) averaged across all treatments when compared to the other selections and ‘Bailey Compact’ (Fig. 3. C1). SW-30-159 may require development of a more efficient rooting method for commercial production. SW-30-14 (66% PR) and SW-30-130 (67% PR) had the highest PR and SW-30-70 was third with (56% PR). SW-30-14, SW-30-130 and SW-30-70 had higher PR then other selections and ‘Bailey Compact’ (46% PR) and will likely perform well in commercial propagation. The overall MNR was lowest with SW-30-159 (0.3 roots per cutting) and SW-30-161 (1.4 roots per cutting). Selections SW-30-14, SW-30-70, SW-30-130 and ‘Bailey Compact’ all had similar MNR of 3.6, 2.3, 3.1 MNR, respectively. Selection SW-30-14 had the numerically highest MRL of the seedless selections (1.7) and was not significantly different from ‘Bailey Compact’ (1.4 in MRL; Fig. 3C3). Selection SW-30-159 and SW-30-161 had the lowest MRL (Fig. 3C3). Root length and number are good indicators of the strength of the rooting system and can be associated with cutting survival and growth potential (Kumar 2016). Our results indicate that seedless selections of Amur maple have different post-mutagenesis capacities for adventitious rooting as measured by PR, MNR and MRL. Relative to ‘Bailey Compact’, seedless selections SW-30-14 and SW-30-130 had higher PR and SW-30-70 similar PR. Commercial propagators will likely find these selections perform well with little adaptation to their current practices. Propagation of SW-30-159 and SW-30-161 was significantly reduced relative to other selections and ‘Bailey Compact’, indicating if these are to be propagated commercially, optimization of propagation may be necessary.

Rooting response of seedless selection with varying auxin type and concentration. Differential PR, MNR and MRL responses were observed with different hormone types and concentrations (Fig. 4). Cuttings treated with 1,500 ppm NAA had the greatest PR except SW-30-159 and ‘Bailey Compact’, although other rooting treatments produced a similar PR to this treatment (Fig. 4C). For MNR and MRL, there was similar diversity among treatments and most measures were not significantly different among auxin treatments. As with PR, SW-30-159 and SW-30-161 had low levels of MNR and MRL relative to other seedless selections and ‘Bailey Compact’ indicating both selections were difficult to root. Survival of cuttings depends on PR and MRN (Kumar 2016). We recommend use of 1,500 ppm

Fig. 5. A) Example of callus formation on an Amur maple cutting (SW-30-161, 100 ppm NAA) without root development. B) Is a magnification of the boxed area shown in A) with extensive callus formation of the stem. The white bar is equal to 1 cm.
NAA for these selections, since this treatment produced similar PR, MNR, and MRL among selections with good rooting competency, and is similar to auxin levels tested by other researchers (Chapman et al. 1979, Gislerød et al. 1983, Dirr et al. 1990).

Callus formation. Soil moisture and temperature of rooting media can impact formation of callus on Amur maple softwood cuttings (Gislerød et al. 1983). Callus production was noted during adventitious rooting and can negatively affect rooting efficiency (Gislerød et al. 1983). Many of the softwood cuttings that produced callus did not develop roots, but the overall health of a cutting with callus was good (Fig. 5). Further research is necessary to evaluate rooting conditions (media moisture and temperature) and its impact on callus versus root formation in Amur maple. Optimization of the environmental conditions could reduce callusing and improve rooting efficiencies of Amur maple selections.

This study demonstrates that seedless selections of Amur maple produced through mutagenesis respond differently to rooting treatments with selections having either greater or lesser competency for rooting than the control ‘Bailey Compact’. Selections SW-30-14 and SW-30-130 had significantly greater PR than ‘Bailey Compact’, however the MNR for selection SW-30-130 was significantly lower than ‘Bailey Compact’. Auxin type and concentration had a significant effect on rooting efficiency of specific seedless selections, agreeing with data from Dirr (1990). The selection SW-30-159 had low rooting potential and may not be suitable for commercial propagation using current methods without additional improvement. Future research should focus on methods of delivering auxin, moisture level of the rooting medium, misting rates and temperature to optimize PR, MNR and MRL for these selections. This optimization (Gislerød et al. 1983) would most likely positively affect rooting efficiency in selections that showed high levels of callus formation (Fig. 5).

Seedless selections of Amur maple produced through mutagenesis respond differently to growth hormone treatments and levels. Results of our study confirm the need to evaluate rooting methods for seedless selections to avoid propagation issues during commercialization and to achieve optimal propagation efficiency. Optimization of rooting methods can significantly enhance propagation efficiency of new Amur maple seedless selections.

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