Development of Micropropagation in Bigleaf Maple (Acer macrophyllum)

Chen Zhou and Jim Mattsson *

Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 0A1, Canada; czhou.bise@gmail.com
* Correspondence: jim_mattsson@sfu.ca

Abstract: Natural populations of bigleaf maple (Acer macrophyllum Pursh) trees contain, at low frequency, individuals with stems that have attractive and valuable wavy grain in the wood. To maintain the genotype of these individuals, vegetative propagation is desired. To enable propagation from the limited amount of plant tissue that is often available, an in vitro micropropagation procedure was developed. A mix of wild trees was used as source material to generate a procedure that is genotype unspecific. Among tested basal media, DKW medium resulted in the highest frequency of growing shoots. For multiplication of shoots, removal of the apex of shoot explants was instrumental, presumably because this treatment broke a strong apical dominance in this species. Of tested hormone and hormone combinations, 0.1 µM thidiazuron produced the best results with an average of 3.2 axillary shoots per explant with an average of 3.7 nodes per axillary shoot after 1 month. Although rooting did not require hormone treatment, a 68% frequency of rooting was obtained on 1/2 MS supplemented with 1 µM IBA, 27% higher than hormone-free media. Taken together, we have developed a procedure for propagation of bigleaf maple from a limited amount of tissues that can be used to multiply various genotypes of interest.

Keywords: TDZ; apical dominance; figured wood

1. Introduction

Bigleaf maple is a large deciduous tree in the genus Acer native to the Pacific west coast of USA and Canada, and the only commercially important maple in the region. As its name implies, this species has large leaves, up to 0.3 m wide [1]. When direct light is available, bigleaf maple grows rapidly [2]. It is classified as a softwood maple and generally used for the manufacturing of furniture. At a low frequency, wild trees also exhibit wood with a variety of figures: curly, quilted, birdseye, and fiddleback [3,4]. The figured wood is highly valued and used to produce veneer, stringed instruments, and gunstocks [5–7]. Figured wood, especially curly wood, is also found in other maple species and deciduous tree species, e.g., birch and walnut. The cause of figured wood is unknown but genetic control is considered as the key factor when extensive figured wood is found in trees [8–10]. While the curly-birch wood phenotype segregates as a monogenic semi-dominant trait [11], there is no evidence of simple Mendelian ratios in other species at this point. Therefore, trees with extensive figured wood are generally propagated by cloning. Evidence for true to form propagation was recently observed when eleven-year-old trees clonally propagated from sycamore maples with wavy grain also began to show wavy grain in stem wood [12,13].

Propagation by cutting is influenced by species, age, and genotype of the donor tree, and may be highly inefficient when using twigs from mature trees with the desired figure as source material [13]. Propagation by rooting of bigleaf maple stem cuttings is inefficient and unreliable [14]. In comparison, in-vitro micropropagation has the potential to produce many new plants from limited starting material, and also to rejuvenate shoots and provide responsive starting material for conventional rooting of cuttings [12].
While a tissue culture method for bigleaf maple is lacking, a few procedures have been published for other maple species [15]. These procedures use nodal segments, shoot tips, and axillary buds as explants. The basal salt composition varies between Murashige and Skoog (MS) medium, woody plant medium (WPM), Linsmaier and Skoog (LS), and Driver and Kuniyaki Walnut (DKW) medium. For most maple species, the cytokinin benzyladenine (BA, also known as 6-benzylaminopurine) is used for shoot induction [15–19]. For sycamore maple (A. pseudoplatanus L.) and Norway maple (A. platanoides L.), cultivar Crimson Sentry, a high BA concentration, can be used [16,17]; whereas for other maple species, high BA concentration results in excessive basal callus formation [17,18]. However, BA is found necessary to promote shoot growth and shoot elongation even for the species that need a low dosage of it [17,19]. The synthetic cytokinin thidiazuron (TDZ) has been tested in the micropropagation of maple species and has been shown to greatly increase shoot multiplication rate [16,17,19,20]. Moreover, TDZ often induce more shoots at lower concentrations than adenine-based cytokinins [15,21].

Since species-to-species differences in the response to media and hormones are considerable, a specific procedure for micropropagation of bigleaf maple is critical before this approach can be used for micropropagation of limited and precious material from the individual trees and logs that harbour valuable figured wood. The objective of this research was, therefore, to test critical parameters to develop a protocol for initiation of micropropagation of bigleaf maple.

2. Materials and Methods

2.1. Plant Material

2.1.1. Shoot Explants from Sprouts of Field-Grown Trees

Sprouts of trees regularly cut under a power line on Burnaby Mountain where Simon Fraser University is located (49.27676° N, −122.918° E) were collected in the spring, washed with water, and placed in buckets with water in the greenhouse. After 1–2 weeks, shoots grew from buds and were used as explants, hereafter referred to as Field Sprouts (FS).

2.1.2. Shoot Explants from 1-Year-Old Greenhouse Grown Plants

Seeds were collected from trees on the north-west side of Burnaby Mountain, germinated and grown for 1 year in a greenhouse (49.27676° N, −122.918° E, hereafter referred to as GP for Greenhouse Plants). Plants were grown in one-gallon pots filled with PRO-MIX soil (Premier Tech Ltd., Rivièr du-Loup, QC, Canada) and watered with 0.5 g/L 20-20-20 fertilizer (Nutrien, Calgary, AB, Canada) once every second week.

2.2. Surface Sterilization

Shoot tip explants, 3–4 cm tall after trimming the leaves, were brushed gently with a toothbrush in soapy water and then rinsed under running tap water for 20 min. The explants were then brought into a laminar air flow hood and sterilized with 70% ethanol for 0.5 to 7 min, then soaked in 10% (v/v) bleach (6% NaOCl, Clorox Co., Brampton, ON, Canada) solution for 2 to 25 min (see results), and subsequently rinsed three times in sterile distilled water.

2.3. Shoot Initiation Media Preparation and Culture Condition

Shoot induction media (SIM) were DKW [22] 1/2 MS [23] or WPM [24] media (Phytotech Labs, Lenexa, KS, USA) supplemented with 3% sucrose, 8 g/L plant agar (Phytotech Labs), three concentrations of BA, and 2.5 g/L activated charcoal (Sigma-Aldrich, Oakville, ON, Canada). Media pH was adjusted to 5.7–5.8 before autoclaving at 121 °C for 20 min. Media were cooled to 60 °C before the addition of 0.1% (v/v) PPM (Plant Preservative mixture, Plant Cell Technology, Washington, DC, USA) and filter-sterilized timentin (GlaxoSmithKline, Mississauga, ON, Canada) to a final concentration of 200 mg/L, then poured into baby-food or GA-7 Magenta vessels (Sigma-Aldrich, Oakville, ON, Canada). After surface sterilization, shoots were dabbed dry on sterile filter paper, the shoot base surface
trimmed-off, and the shoot inserted in the above media. Batches of >30 explants collected from a range of genotypes were processed at a time. All the cultures were kept in a plant incubator, programmed to 22 °C with a 16-h photoperiod and 90% humidity under a light intensity of 28 µmol m⁻² s⁻¹ provided by cool-white fluorescent tubes. Data on shoot growth initiation were collected after 1 month.

2.4. Shoot Multiplication

The apices of shoots established on SIM were cut off, leaving at least one node on each segment. Apex and basal portion were cultured on DKW media supplemented with a series of hormone combinations: BA, BA with NAA (1-naphthaleneacetic acid), and TDZ with NAA (Table 1). Hormones were purchased from Phytotech Labs (Lenexa, KS, USA). Explants were also treated with TDZ alone in four concentrations: 0.01, 0.05, 0.1, and 1 µM. After 1 month, newly proliferated shoots were removed from the media and subcultured on DKW medium supplemented with 0.01, 0.05, or 0.1 µM TDZ. At this point, the shoot response rate (axillary bud sprouting rate), the number of microshoots, their length and number of nodes were recorded. Microshoot length was measured from the apical meristem to the base, callus not included.

Table 1. Effects of hormone combinations on multiplication of decapitated shoots from greenhouse plants after 2 months.

| BA (µM) | NAA (µM) | TDZ (µM) | No. Expl. | Resp. (%) | No. Shoots | Axillary Shoot Length (cm) | Nodes per Axillary Shoot |
|---------|----------|----------|-----------|-----------|------------|--------------------------|------------------------|
| 0.44    | -        | -        | 31        | 39        | 1.1 ± 0.2f | 0.67 ± 0.08g             | 1.3 ± 0.1g             |
| 1.33    | -        | -        | 31        | 52        | 1.7 ± 0.1c | 1.11 ± 0.07cd             | 2.1 ± 0.1cde           |
| 2.22    | -        | -        | 28        | 61        | 2.3 ± 0.1ab| 1.38 ± 0.13ab             | 2.5 ± 0.1ab            |
| 4.44    | -        | -        | 32        | 41        | 1.3 ± 0.1e | 0.80 ± 0.06fg             | 1.7 ± 0.1fg            |
| 1.33    | 0.054    | -        | 34        | 59        | 1.8 ± 0.1c | 1.03 ± 0.06def            | 2.2 ± 0.1cde           |
| 1.33    | 0.27     | -        | 36        | 64        | 2.2 ± 0.1ab| 1.29 ± 0.06abc            | 2.6 ± 0.1a             |
| 1.33    | 0.54     | -        | 36        | 47        | 1.4 ± 0.1e | 0.81 ± 0.06fg             | 1.8 ± 0.1efg           |
| -       | 0.27     | 0.01     | 32        | 47        | 1.5 ± 0.1de| 0.88 ± 0.09efg            | 1.8 ± 0.1def           |
| -       | 0.27     | 0.05     | 33        | 58        | 2.1 ± 0.1bc| 1.19 ± 0.08bcd            | 2.2 ± 0.2bc            |
| -       | 0.27     | 0.1      | 35        | 69        | 2.4 ± 0.1a | 1.40 ± 0.06a              | 2.9 ± 0.1a             |

Abbreviations: BA (benzyl adenine), NAA (1-naphthaleneacetic acid), TDZ (thidiazuron), expl. (explants), resp. (response by growth). Values represent means ± standard error of the mean (SEM). Means within columns followed by different letters are significantly different by Fishers LSD, \( p < 0.05 \).

2.5. In Vitro Rooting

Shoots longer than 1 cm were excised and placed on \( 1/2 \) MS supplemented with different indole-3-butyric acid (IBA) concentrations.

2.6. Ex Vitro Acclimatization

Well-rooted plants were removed from agar media and roots were gently washed with sterilized distilled water and transferred to wet vermiculite (Premier Tech Ltd., Rivière-du-Loup, QC, Canada) in GA-7 vessels (Sigma-Aldrich, Oakville, ON, Canada) with a second vessel placed on top without seal. After 1–2 weeks, plantlets were potted in PRO-MIX peat mix (Premier Tech Ltd., Rivière-du-Loup, QC, Canada) watered to field capacity with 0.5 g/L 20-20-20 fertilizer and kept in propagation trays with transparent lids in a growth chamber with intermittent mist. The lids of the containers were opened slightly after 2 days and then wider in the following days until being removed, acclimating plants to lower humidity. Plants were grown for 1 month before re-potting in one-gallon pots and transferred to a greenhouse.

2.7. Statistical Design and Analysis

Seeds and sprouts were collected from a wide range of wild bigleaf maple trees, and explants collected from across available plants to reduce genotype-specific variation.
in responses. Similarly, vessels with different hormone and media compositions were distributed randomly under the growth lights to minimize the influence of local variation in growth conditions on responses. Data sets were subjected to two-way analysis of variance (ANOVA) and means were separated by a Fisher’s least significant difference (LSD) test using the JMP13 software (SAS).

3. Results

3.1. Surface Sterilization

As expected, the contamination rate declined as the duration in 70% (v/v) ethanol and 10% (v/v) bleach increased. However, rate of tissue browning also increased and subsequent lack of shoot growth was caused by longer bleach or ethanol treatments. When axillary shoots were collected directly in the field from young trees and sprouts, the highest survival rate was 25%, using a 0.5-min treatment with 70% (v/v) ethanol, followed by a 10-min treatment with 10% (v/v) bleach and 0.5-min ethanol treatments (Table S1). This low rate was improved by bringing sprouts into the greenhouse before bud burst, presumably due to the reduction in the load of microbes. When such shoots were used, the most successful trade-off was a 1-min treatment with 70% (v/v) ethanol, followed by 10% (v/v) bleach for 5 min with a survival rate of 47%, as 5% of shoots succumbed to browning and 48.1% to infection (Table S2). For shoots taken from Greenhouse Plants (GP), the most successful trade-off between contamination and browning was a 30-s treatment with 70% (v/v) ethanol followed by 10% (v/v) bleach for 25-min, resulting in a survival rate of 79% (Table S3). Given the 30% higher survival rate of shoots from greenhouse plants and year-round availability, they were used the most in subsequent experiments.

3.2. Shoot Growth Induction

3.2.1. Preliminary Tests

Initial experiments showed that regardless of hormone combinations, media containing activated charcoal resulted in a 15% to 24% higher frequency of growing shoots than media without activated charcoal (data not shown). Tests with BA at concentrations of 2.2 µM or 4.4 µM, previously used for Manitoba maple (Acer negundo) [25], promoted growth of buds and shoots (Figure 1a). Tests with zeatin at the 5 to 27 µM concentrations used for bigtooth maple [26] promoted shoot growth but also induced extensive callus formation (Figure 1c). Similar results were obtained when combinations of zeatin and TDZ were tested (Figure 1b,d). An initial experiment also showed that the frequency of shoot browning was markedly higher on MS-containing media (75%) than 1/2 MS (40%), DKW (17%), or WPM (0%) media. Based on these results, activated charcoal was included in all shoot initiation media, BA was used for initiation of shoot growth, and media with DKW, WPM, and 1/2 MS salts were compared. None of these treatments resulted in proliferation of axillary shoots from the base of petioles, an issue addressed in 3.3.3.
Figure 1. Results from initial experiments to induce shoot growth in bigleaf maple on DKW medium. BA (2.2 μM)—induced bud (a). Shoot grown on medium supplemented with 10 μM zeatin and 0.01 μM TDZ (b). A shoot cultured on 27 μM zeatin produced a large callus base with multiple petioles and no additional shoots (c). Example of rare axillary shoot proliferation in the absence of shoot tip removal, on media containing 10 μM and 0.01 μM TDZ zeatin (d).

3.2.2. Basal Media and BA Concentrations Experiments

Use of DKW basal medium resulted in a more than 30% higher rate of growing shoots than 1/2 MS and WPM media (Figure 2). A comparison of BA concentration on shoot growth showed that 2.2 μM BA induced a more than 25% higher rate of growing shoots than hormone-free medium, and also that higher BA concentrations (4.4 and 6.6 μM) failed to increase the frequency of shoots that grew (Figure 3).

Figure 2. The percentage of shoot apices taken from greenhouse plants (GP) and field sprouts (FS) that grew in response to the treatments of three basal media DKW (green), 1/2 MS (orange), and WPM (blue) and two different concentrations of BA (μM). Total number of tested shoot apices from left to right: 54, 36, 35, 72, 39, 40, 39, 43, 42, 62, 37, 41. Error bars represent standard error. DKW medium resulted in significantly higher frequency of growing shoots than 1/2 MS and WPM media, as indicated by different letters above bars (two-way ANOVA and Fishers LSD, p < 0.05).
Figure 3. The frequency of growing shoot apices taken from greenhouse plants (GP; green) and field sprouts (FS; orange) shoots of bigleaf maple to three concentrations of BA in DKW basal medium. Total number of tested shoot apices from left to right: 54, 48, 54, 72, 39, 62, 50, 27. Error bars represent standard error. Medium with 2.2 or 4.4 μM BA resulted in significantly higher frequency of growing shoots than medium without BA, as indicated by different letters above bars (two-way ANOVA and Fishers LSD, \( p < 0.05 \)).

3.2.3. Shoot Proliferation
Initial Exploratory Experiments

In the previous experiments, few explants showed growth of shoots from axillary buds. Similarly, exposure to the potent synthetic cytokinin TDZ (0.01 μM), resulted in only approximately 4% of explants with axillary shoot growth. Higher cytokinin concentrations did not increase axillary shoot growth and instead resulted in massive callus formation at the base and, with time, also the conversion of newly formed shoots into calli (data not shown). We observed, however, that removal of the apex from elongated shoots promoted axillary shoot growth and, therefore, tested this treatment in combination with various concentrations of cytokinins.

Hormone Combination Screen on Shoots without Apices

To test the effect of removing the shoot tip on axillary shoot development, shoots from greenhouse plants were surface-sterilized, cut into an apical and basal segment, and cultured side-by-side on shoot-inducing media. After 1 month, the apical segments had typically grown and produced additional leaves but no axillary shoots (the left shoots in Figure 4c,d; leaves removed). On the other hand, the basal segments had produced strong axillary shoots (right shoots in Figure 4c,d) and, for some axillary shoots, additional shoots also emerged from the axils of leaves (not shown). At a low frequency, adventitious shoots also appeared from the callus base of the apical part of the shoots (Figure 4a).
Figure 4. The effect of apex removal (decapitation) on shoot multiplication. Multiple shoots developed on DKW medium supplemented with 0.1 (a) or 0.01 (b) µM TDZ and grown for 1 month. The right shoot in (b) was split into an apical and basal segment and cultured side by side on medium with 0.01 µM TDZ. After 1 month, the apical shoot had produced leaves (removed for clarity) but no axillary shoots (c), whereas the basal segment had produced multiple axillary shoots (arrows in c). The same treatment of the left shoot in (b) had the same outcome (d). Red asterisks in (a) indicates shoots formed de novo from callus.

(A) Effect of BA, BA+NAA and TDZ+NAA on shoots from greenhouse plants after decapitation

To systematically assess the effect of hormones on axillary shoot proliferation, the hormone combinations and concentrations listed in Table 1 were tested. Of the ten tested treatments, three resulted in a significantly higher number of axillary shoots, length of new shoots, and number of nodes per axillary shoot: 2.2 µM BA, 1.33 µM BA combined with 0.27 µM NAA, or 0.1 µM TDZ combined with 0.27 µM NAA. The three treatments resulted in 2.2 to 2.4 axillary shoots per explant, axillary shoot lengths of 13 to 14 mm, and 2.5 to 2.9 nodes per axillary branch.

(B) Effect of TDZ on shoots from greenhouse plants after decapitation

To assess the effect of TDZ alone, four concentrations of TDZ were tested. Treatment with 0.1 µM TDZ resulted in a significantly higher number of axillary shoots, longer shoots, and more nodes per shoot than those obtained on media with 0.01, 0.05, and 1.0 µM TDZ (Table 2). The number of new shoots per explant increased with increasing TDZ concentration, reaching a maximum of 3.2 new shoots per explant at 0.1 µM TDZ. One µM TDZ induced fewer shoots, massive proliferation of callus from the base, and conversion of newly formed shoots into calli. The use of 0.01 µM TDZ allowed an inter-experiment assessment of the effect of removing the apex, with 4% response (axillary shoot growth) rate without removal of the apex, compared to 69% in shoots without apices. TDZ at 0.1 µM resulted in an average of 84% of shoots producing axillary shoots, which is 15% higher than the previous best result.

To assess the effect of repeated shoot propagation, shoots from the first round in Table 2 were decapitated and the basal segments cultured on fresh media with the same TDZ concentrations. Shoots grown on 1.0 µM TDZ were excluded because of the clear...
negative effects of this high concentration. As in the first round, shoots treated with 0.1 µM TDZ produced significantly higher number of axillary shoots, longer shoots, and more nodes on new shoots, compared to shoots on 0.01 and 0.05 µM TDZ (Table 2). From a statistical perspective, round 2 produced the same results as round 1, although there was a trend of higher average numbers in round 2.

Table 2. Effect of TDZ concentrations on the multiplication of shoots from decapitated shoots taken from greenhouse-grown plants and field sprouts after 1 month of culture (round 1), and on the multiplication of newly proliferated shoots dissected from round 1 (round 2).

| TDZ (µM) | No. Expl. | Resp. (%) | No. Axillary Shoots | Axillary Shoot Length (cm) | Nodes per Axillary Shoot |
|----------|-----------|-----------|---------------------|-----------------------------|--------------------------|
|          |           |           |                     |                             |                          |
|          | greenhouse explants, round 1 | | | | |
| 0.01     | 32        | 69        | 1.9 ± 0.2c          | 1.06 ± 0.07c                | 2.2 ± 0.2c               |
| 0.05     | 36        | 67        | 2.6 ± 0.3b          | 1.33 ± 0.06b                | 2.7 ± 0.1b               |
| 0.1      | 43        | 84        | 3.2 ± 0.2a          | 1.81 ± 0.06a                | 3.7 ± 0.1a               |
| 1        | 26        | 39        | 4.4 ± 0.2d          | 0.60 ± 0.05d                | 1.7 ± 0.2d               |
|          | greenhouse explants, round 2 | | | | |
| 0.01     | 40        | 63        | 2.4 ± 0.2c          | 1.45 ± 0.08c                | 3.1 ± 0.1c               |
| 0.05     | 36        | 64        | 3.0 ± 0.2b          | 1.66 ± 0.07b                | 3.7 ± 0.1b               |
| 0.1      | 43        | 79        | 3.5 ± 0.2a          | 2.21 ± 0.06a                | 4.8 ± 0.1a               |
|          | field sprout explants, round 1 | | | | |
| 0.01     | 27        | 63        | 2.3 ± 0.3b          | 0.92 ± 0.04b                | 2.5 ± 0.2b               |
| 0.05     | 30        | 67        | 2.4 ± 0.3b          | 1.01 ± 0.06b                | 2.8 ± 0.1b               |
| 0.1      | 29        | 83        | 3.9 ± 0.3a          | 1.31 ± 0.05a                | 3.5 ± 0.2a               |

Abbreviations: TDZ (thidiazuron), expl. (explants), resp. (response by growth). Values represent means ± SEM. Mean followed by different letters within columns are significantly different by Fishers LSD, *p* < 0.05.

(C) Effect of TDZ on shoots from field sprouts after decapitation

Like explants from greenhouse plants, shoots from field sprouts (FS) also produced a significantly higher number of axillary shoots, longer shoots, and more nodes per shoot when exposed to 0.1 µM TDZ, relative to 0.01 and 0.05 µM TDZ (Table 2). Shoots taken from field sprouts and greenhouse explants produced a comparable number of axillary shoots (3.5 versus 3.9 shoots per explant).

3.2.4. Rooting of Shoots

A half concentration of MS salts in combination with IBA is commonly used for rooting of maple shoots [16,27,28]. Therefore, 1/2 MS was tested for rooting of axillary shoots multiplied from field sprouts and greenhouse grown plants in combination with a series of IBA concentrations (Table 3). The highest average rooting frequency was in both cases seen with 1 µM IBA, although the effect was significantly different from hormone-free media only from greenhouse plant-derived shoots. Use of 1 µM IBA resulted in significantly longer roots than any of the other tested IBA concentrations, both from field sprout and greenhouse plant-derived shoots.

Table 3. Effect of IBA concentration on rooting of axillary shoots produced in vitro after one and two rounds of multiplication. Medium contained 1/2 MS salts.

| IBA (µM) | No. Expl. | Rooting (%) | No. Roots/Shoot | Lateral Roots | Root Length (cm) |
|----------|-----------|-------------|-----------------|---------------|------------------|
|          |           |             |                 |               |                  |
|          | rooting after 1st round of multiplication (explants originally from field sprouts) | | | | |
| 0        | 28        | 54          | 1.3             | 0             | 2.49 ± 0.17d     |
| 0.25     | 25        | 52          | 1.9             | 1             | 3.26 ± 0.29c     |
| 0.5      | 27        | 56          | 2.5             | 2             | 4.54 ± 0.09b     |
| 1        | 28        | 64          | 2.7             | 6             | 7.15 ± 0.29a     |
| 5        | 25        | 40          | 2.9             | 3             | 3.73 ± 0.14c     |
Table 3. Cont.

| IBA (μM) | No. Expl. | Rooting (%) | No. Roots/Shoot | Lateral Roots | Root Length (cm) |
|----------|-----------|-------------|-----------------|---------------|-----------------|
|          |           |             |                 |               | Rooting after 2nd round of multiplication (explants originally from greenhouse plants) |
| 0        | 22        | 41          | 2               | 0             | 2.05 ± 0.10d    |
| 0.25     | 42        | 52          | 2.1             | 2             | 4.33 ± 0.26c    |
| 0.5      | 32        | 59          | 2.4             | 6             | 5.42 ± 0.28b    |
| 1        | 37        | 68          | 2.8             | 4             | 6.67 ± 0.24a    |
| 5        | 31        | 45          | 3.4             | 1             | 3.86 ± 0.56c    |

Abbreviations: IBA (indole-3-butyric acid); no. expl., number of tested explants, rooting, percentage of shoots with roots; no. roots/shoot, number of roots per shoot with roots; lateral roots, number of shoots with lateral roots. Values represent means ± SEM. Means within columns followed by different letters are significantly different as determined by Fisher’s LSD, \( p < 0.05 \).

3.2.5. Acclimatization

To provide a gradual acclimation to greenhouse conditions, plantlets with established root systems (Figure 5a,b) were first transferred to wet vermiculite in vessels with lids without seals and kept in a growth chamber for 1–2 weeks (Figure 5c). Thereafter, the plantlets were potted in peat-based mix, covered, and incubated in a growth chamber. After gradual removal of covers and 1 month of growth, a survival rate of 75% was observed (\( n = 55 \), Figure 5d). When these plants were repotted in larger pots and grown in a greenhouse for 2 months, an 83% survival rate was observed, with many plants showing rapid growth (Figure 5e).

4. Discussion

In the vast majority of published procedures, treatment with exogenous cytokinin is sufficient to trigger growth and multiplication of axillary buds [29]. However, for some species, exposure to cytokinins is insufficient to counteract the effect of apical dominance [30–32]. We had the same problem when attempting in vitro propagation of bigleaf maple.
maple. After multiple trials with shoots on media with various cytokinins, concentrations and combination with auxin, there were, with few exceptions, a lack of growth from axillary buds. However, when the shoot apex was removed (decapitated shoots), axillary buds grew into well-developed shoots. In publications on micropropagation of maples, removal of shoot apices was used to trigger outgrowth of axillary buds in cultures of silver maple [21] and snakebark maple [27]. Presumably, the inhibitory effect of the apex on axillary shoot development is an example of apical dominance mediated by auxin produced by the shoot apex and transported basipetally via polar auxin transport [33,34]. The strong apical dominance in tissue culture is in accordance with the strong monopodial growth of bigleaf maple [35]. Of the fourteen tested cytokinin-containing media, DKW supplemented with 0.1 µM TDZ resulted in the highest number of growing primary shoots, and the highest number of axillary secondary shoots, nodes per axillary shoot, and axillary shoot length (Tables 1 and 2). The number of shoots produced per explant in bigleaf maple (3.5 to 3.9 on 0.1 µM TDZ) is in the same range as those observed for bigtooth maple (A. grandidentatum, Nutt), sycamore maple, and red maple (A. rubrum, L) [18,26] and slightly below that for silver maple [21], indicating that obtained results may be close to what can be achieved in this species under tested conditions.

Since each produced axillary shoot had on average 3.5 to 4.8 nodes (0.1 µM TDZ, Table 2) and the shoots were of good quality without vitrified leaves even after repeated multiplication, scaling up clonal production of plants should be possible. In addition, the results were obtained using explants from a mix of wild genotypes shoots. Although the procedure may be suboptimal for specific genotypes, it should yield results when applied to a range of bigleaf maple individuals that produce figured wood and also serve as a starting point for genotype-specific optimization of propagation. In micropropagation of many wood plant species, repeated subcultures result in more stable and homogenous cultures. Such stabilized shoot cultures may respond better to plant growth regulators than earlier shoot cultures, resulting in a higher rate of shoot production [36–38]. In silver maple, a four-fold increase in shoot numbers was seen from the third to the fourth month of subculture [21,39], and it is possible that similar results can be obtained for bigleaf maple. On the other hand, TDZ can accumulate in tissues with time, resulting in various shoot abnormalities and high frequency of somaclonal variation [40], indicating a potential offset between propagation time and quality of resulting plants.

DKW basal medium resulted in a more than 30% higher rate of growing shoots than 1/2 MS and WPM media. A similar comparison for bigtooth maple also obtained the highest growth responses using DKW medium [26]. DKW medium has higher molar levels of several macro ions than 1/2 MS and WPM media [41] (Table S4), which may therefore be the cause of the higher frequency of growth in bigleaf maple, and also potential targets for further optimization of growth responses.

In summary, we tested out parameters for in vitro micropropagation of bigleaf maple. Removal of the shoot tip from explants was required to release growth of axillary shoots and, in combination with the use of DKW medium and 0.1 µM TDZ, resulted in a three to four-fold increase of shoots per month. Rooting frequency was highest on 1/2 DKW supplemented with 0.25 µM IBA, and stepwise reduction of humidity allowed efficient acclimation to greenhouse conditions.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7070170/s1, Table S1: Results of surface sterilization of axillary shoots taken directly from field-grown sprouts, Table S2: Results of surface sterilization of greenhouse-grown axillary shoots derived from field-grown sprouts, Table S3: Results of surface sterilization of greenhouse-grown seedling-derived shoot explants, Table S4: Concentration of macro ions (mM) that differ the most between tested basal media.

**Author Contributions:** Conceptualization, J.M.; Data curation, J.M. and C.Z.; Formal analysis, C.Z.; Funding acquisition, J.M.; Investigation, C.Z.; Methodology, C.Z.; Project administration, J.M.; Resources, C.Z.; Software, C.Z.; Supervision, J.M.; Validation, J.M. and C.Z.; Visualization, C.Z.;
Writing—original draft, J.M. and C.Z.; Writing—review and editing, J.M. and C.Z. Both authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Pacific Rim Tonewoods Ltd. Concrete, WA, U.S., and by a discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC RGPIN-2020-06918 to Jim Mattsson). We thank Steven McMinn and Kevin Burke of Pacific Rim Tonewoods Ltd. for providing funding for this project and for providing figured bigleaf maple wood samples used in this project. This project would not have been possible without their generous support.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** We are indebted to the three anonymous reviewers and journal staff that provide constructive criticism that greatly improved this manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Iddrisu, M.N.; Ritland, K. Genetic variation, population structure, and mating system in bigleaf maple (*Acer macrophyllum* Pursh). *Can. J. Bot.* 2004, 82, 1817–1825. [CrossRef]
2. Peterson, E. Bigleaf Maple Managers’ Handbook for British Columbia; Technical Report; British Columbia, Ministry of Forests Research Program: Terrace, BC, Canada, 1999.
3. Panshin, A.J.; De Zeeuw, C. Textbook of Wood Technology; McGraw-Hill: New York, NY, USA, 1980.
4. Beals, H.O.; Davis, T.C. *Figure in Wood: An Illustrated Review*; Auburn University: Auburn, AL, USA, 1977.
5. Riede, A. Ahorn-Werholzproduktion in kurzen Umtrieben. *AFZ Der Wald* 1998, 15, 776–779.
6. Krajnc, L.; Cufar, K.; Brus, R. Characteristics and Geographical Distribution of Fiddleback Figure in Wood of *Acer pseudoplatanus* L. in Slovenia. *Drv. Ind.* 2015, 66, 213–220. [CrossRef]
7. Kobal, M.; Kristan, S.; Grudnik, P.; Vilhar, U. Supply and demand at auctions of value wood assortments in Slovenj Gradec. *Gozdarski Vestn.* 2013, 71, 462–470.
8. Fan, Y.; Rupert, K.; Wiedenhoeft, A.C.; Woeste, K.; Lexer, C.; Meilan, R. Figured grain in aspen is heritable and not affected by graft-transmissible signals. *Trees Struct. Funct.* 2013, 27, 973–983. [CrossRef]
9. Persson, A. Seed orchard production of figured birch. *Skogen* 1954, 41, 160–163.
10. Václavev, E. The quality of wood of the technical forms of birch. In Proceedings of the Second World Consultation on Forest Tree Breeding, Washington, DC, USA, 7–16 August 1969; FAO: Rome, Italy, 1970; Volume 1, pp. 437–446.
11. Kärkkäinen, K.; Viherä-Aarnio, A.; Vakkari, P.; Hagqvist, R.; Nieminen, K. Simple inheritance of a complex trait: Figured wood in curly birch is caused by one semi-dominant and lethal Mendelian factor? *Can. J. For. Res.* 2017, 47, 991–995. [CrossRef]
12. Ewald, D.; Naujoks, G. Vegetative propagation of wavy grain *Acer pseudoplatanus* and confirmation of wavy grain in wood of vegetatively propagated trees: A first evaluation. *Dendrobiology* 2015, 74, 135–142. [CrossRef]
13. Rohr, R.; Hanus, D. Vegetative propagation of wavy grain sycamore maple. *Can. J. For. Res.* 1987, 17, 418–420. [CrossRef]
14. Harrington, C.A.; McGrath, J.M.; Kraft, J.M. Propagating Native Species: Experience at the Wind River Nursery. *West. J. Appl. For.* 1999, 14, 61–64. [CrossRef]
15. Đurkovič, J.; Mišalová, A. Micropropagation of temperate noble hardwoods: An overview. *Funct. Plant Sci. Biotechnol.* 2008, 2, 1–9.
16. Lattier, J.D.; Touchell, D.H.; Ranney, T.G. Micropropagation of *Acer platanoides* L. “Crimson Sentry.” *SNA Res. Conf.* 2012, 57, 296–300.
17. Wilhelm, E. Micropropagation of juvenile sycamore maple via adventitious shoot formation by use of thidiazuron. *Plant Cell Tissue Organ Cult.* 1997, 57, 57–60. [CrossRef]
18. Wann, S.R.; Gates, E.E. Micropropagation of mature red maple (*Acer rubrum* L.). In Proceedings of the Southern Forest Tree Improvement Conference, Atlanta, Georgia, 14–17 June 1993; pp. 99–105.
19. Đurkovič, J. In vitro regeneration of Norway maple (*Acer platanoides* L.). *Biol. Plant.* 1996, 38, 303–307. [CrossRef]
20. Kerns, H.R.; Meyer, M.M., Jr. Diligence finds the chemical key to micropropagating a new maple. *Am. Nurserym.* 1987, 165, 104–105.
21. Preece, J.E.; Huetteman, C.A.; Ashby, W.C.; Roth, P.L. Micro-and Cutting Propagation of Silver Maple. I. Results with Adult and Juvenile Propagules. *J. Am. Soc. Hortic. Sci.* 1991, 116, 142–148. [CrossRef]
22. Driver, J.; Kuniyuki, A. In vitro propagation of Paradox walnut rootstock. *HortScience* 1984, 19, 507–509.
23. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* 1962, 15, 473–497. [CrossRef]
24. Lloyd, G.; McCown, B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *Int. Plant Propagators’ Soc. Proc.* 1980, 30, 421–427.
25. Dou, Y. The Tissue Culture of Two Maple and Preliminary Research on Prevention of Tissue Pollution. Master’s Thesis, Liaoning Normal University, Dalian, China, 2010.

26. Bowen-O’Connor, C.A.; Hubstenberger, J.; Killough, C.; VanLeeuwen, D.M.; St. Hilaire, R. In vitro propagation of Acer grandidentatum Nutt. In Vitro Cell. Dev. Biol. Plant 2007, 43, 40–50. [CrossRef]

27. Đurković, J. Regeneration of Acer caudatifolium Hayata plantlets from juvenile explants. Plant Cell Rep. 2003, 21, 1060–1064. [CrossRef]

28. Marks, T.R.; Simpson, S.E. Factors affecting shoot development in apically dominant Acer cultivars in vitro. J. Hortic. Sci. 1994, 69, 543–551. [CrossRef]

29. George, E.F.; Hall, M.A.; De Klerk, G.-J. Plant growth regulators I: Introduction; auxins, their analogues and inhibitors. In Plant Propagation by Tissue Culture; Springer: Heidelberg/Berlin, Germany, 2008; pp. 175–204.

30. Bhojwani, S.S.; Dantu, P.K. Plant Tissue Culture: An Introductory Text; Springer: New Delhi, India, 2013.

31. Chaturvedi, R.; Razdan, M.K.; Bhojwani, S.S. An efficient protocol for the production of triploid plants from endosperm callus of neem, Azadirachta indica A. Juss. J. Plant Physiol. 2003, 160, 557–564. [CrossRef]

32. Dun, E.A.; Ferguson, B.J.; Beveridge, C.A. Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiol. 2006, 142, 812–819. [CrossRef] [PubMed]

33. Cline, M.G. Concepts and terminology of apical dominance. Am. J. Bot. 1997, 84, 1064–1069. [CrossRef] [PubMed]

34. Taiz, L.; Zeiger, E.; Møller, I.M.; Murphy, A.S. Plant Physiology and Development; Sinauer Associates: Sunderland, MA, USA, 2015.

35. Savidge, R.A. Tree growth and wood quality. Wood Qual. Biol. Basis 2003, 240, 1–29.

36. Kristiansen, K. Micropropagation of Ficus benjamina clones. Plant Cell Tissue Organ Cult. 1992, 28, 53–58. [CrossRef]

37. Grant, N.J.; Hammatt, N. Increased root and shoot production during micropropagation of cherry and apple rootstocks: Effect of subculture frequency. Tree Physiol. 1999, 19, 899–903. [CrossRef] [PubMed]

38. Harris, R.A.; Mantell, S.H. Effects of Stage II subculture durations on the multiplication rate and rooting capacity of micropropagated shoots of tree paeony (Paonia suffruticosa Andr.). J. Hortic. Sci. 1991, 66, 95–102. [CrossRef]

39. Huetteman, C.A.; Preece, J.E. Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell Tissue Organ Cult. 1993, 33, 105–119. [CrossRef]

40. Dewir, Y.H.; Nurmansyah; Naidoo, Y.; Teixeira da Silva, J.A. Thidiazuron-induced abnormalities in plant tissue cultures. Plant Cell Rep. 2018, 37, 1451–1470. [CrossRef] [PubMed]

41. Bell, R.L.; Srinivasan, C.; Lomberk, D. Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cell. Dev. Biol. Plant 2009, 45, 708–714. [CrossRef]