Histology of Adventitious Root Formation and Phytohormone Analysis of American Chestnut Cuttings[1]

Xinya Lu, Maxine Cuarto, and Haiying Liang[2]*

Abstract

The formation of adventitious roots (ARs) is a complex process. It plays an important role in the successful production of elite clones since it is a key step in the vegetative propagation of economically important horticultural woody species. The American chestnut (*Castanea dentata*) is a heritage species and is notoriously recalcitrant to stem rooting. As part of the efforts to understand American chestnut cuttings' recalcitrance, we examined AR formation via histology and compared the phytohormone level profile between American chestnut and easy-to-root poplar cuttings (*Populus x euramericana*). It was found that ARs could be induced directly from American chestnut cuttings without callus formation. Adventitious roots of American chestnut were initiated from cambial derivatives and developed a vascular system connected with that of the stem. Compared to easy-to-root poplar, American chestnut cuttings had a low level of indole-3-acetic acid (IAA) and a high level of cytokinin (CK), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and oxylipin 12-oxo-phytodienoic acid (OPDA). Hormone distribution between leaves and stems also differed between American chestnut and poplar. This unfavorite endogenous hormone profile may contribute to American chestnut cuttings' recalcitrance to rooting.

Species used in this study: American chestnut [*Castanea dentata* (Marsh.) Borkh.], poplar (*Populus x euramericana*).

Chemicals used in this study: 1-Naphthaleneacetic acid (NAA).

Index words: Phytohormones, propagation, vegetative cuttings.

Significance to the Horticulture Industry

The American chestnut was among the largest, tallest, and fastest-growing trees in the eastern United States, with great economic and ecological value. The species is notoriously recalcitrant to stem rooting. Overcoming this obstacle by developing an efficient cutting rooting system for this heritage species is critical for the rapid and mass production of elite cultivars. This study identifies the location and cell type that form American chestnut root primordia and reveals the differences in endogenous hormones between American chestnut and easy-to-root poplar cuttings. It is suggested that a low auxin content and high levels of root-inhibiting endogenous hormones, such as ABA, JA, SA, and cytokinins contribute to American chestnut's recalcitrance to rooting. This work provides novel insights into American chestnut's adventitious root induction, laying the foundation for improving the rooting system in American chestnut cuttings. The impact is of great significance to the restoration and diversity conservation of the species.

Introduction

Plant propagation can be sexual or asexual (vegetative or clonal). Because sexual propagation involves the recombination of parental genomes and traits, genetic variation is introduced, while for asexual propagation, new plants are produced from vegetative parts of the original plant, such as stems, roots, buds, and leaves. Therefore, asexually propagated plants are genetically identical to the original plant and are homogeneous, ensuring the consistency of high-quality plants. Among the many types of vegetative propagation (cuttings, grafting, budding, layering, stooling, micropropagation, etc.), rooting of stem cuttings is the most used method to maintain desired genotypes in various woody species, due to its features such as ease of operation and low skill requirement. However, some species are notoriously recalcitrant, including *Castanea dentata*, the American chestnut.

The American chestnut was among the largest, tallest, and fastest-growing trees in the eastern United States, covering up to 45% of the forest canopy in some areas (Keever 1953). Rich in carbohydrates and other nutrients, chestnuts were an excellent food source for thousands of years, feeding wildlife, people, and their livestock. The wood was rot-resistant, straight-grained, and suitable for furniture, fencing, and building. The species was a good source of tannins and had the ability to quickly colonize burned or clearcut areas. Because of its ecological and economic importance, the American chestnut was referred to as the "perfect tree" (Freinkel 2007).

Unfortunately, due to the accidental introduction of the exotic disease chestnut blight [caused by *Cryphonectria parasitica* (Murr.) Barr], in the early 1900s, the American chestnut is now widely regarded as "functionally extinct", as only a small percentage of these trees reach sexual maturity. Known as the greatest ecological disaster to strike the world's forests in history, the chestnut blight, within 40 years, virtually eliminated the American chestnut trees that once dominated the eastern half of the U.S. and had survived all adversaries for 40 million years (Brewer 1995). Another exotic disease, Phytophthora root rot (caused by *Phytophthora*

Received for publication December 24, 2022; in revised form April 7, 2023.

[1]The project was jointly funded by the Horticultural Research Institute and the American Chestnut Foundation.

[2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC, 29634, 105 Collings Street, Biosystems Research Complex, Room 108, Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, Telephone: 864-656-2414.

*Corresponding author email: hliang@clemson.edu.

 Copyright 2023 Horticultural Research Institute

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

cinnamomi Rands), was introduced in the 1700s and is thought to have eradicated American chestnut trees from low-elevation forests in the Southeastern U.S. prior to the introduction of chestnut blight (Anagnostakis 2012).

Because of the great economic and ecological value of the American chestnut tree, significant efforts have been made over the century to combat the above-mentioned diseases with an aim to restore the iconic chestnut to American forests. To date, hybridizing with a blight-tolerant chestnut species followed by repeated back-crossing (Steiner et al. 2017, Clark et al. 2019) and genetic engineering (Newhouse et al. 2014) have generated promising blight-tolerant varieties. Once the disease-tolerant varieties become available, a rapid and low-cost method for clonal reproduction is needed to ensure the success of the American chestnut restoration efforts.

Studies in other species show that there are four stages for forming adventitious roots (ARs): 1) dedifferentiation of parenchyma cells in the phloem ray area, 2) formation of root initials, 3) formation of the root primordia, and 4) elongation (Hartmann et al. 2010). In woody species such as poplar and apple [*Malus domestica* (Suckow) Borkh.], AR can develop from stem pericycle-like tissue adjacent to the vascular bundles or from the calli that differentiate after wounding (Gonin et al. 2019), while no similar reports are available in American chestnut cuttings. As part of the efforts to fill the research gap and attempt to understand why American chestnut cuttings are difficult to root, we examined AR formation via histology and compared the phytohormone level profile between American chestnut and easy-to-root poplar cuttings. Such information is essential for overcoming this recalcitrance and for developing an efficient cutting rooting system for the heritage species.

Materials and Methods

AR induction in American chestnut cuttings. Current-year semi-lignified shoots were clipped from juvenile American chestnut seedlings in June 2022. Shoots were cut to 10-15 cm (4-6 in), with two or three leaves, whose size was reduced to approximately 5 cm (2 in) in length. The bottom of the cuttings (approximately 2 cm, 0.8 in), which included at least 1 node, was dipped in 0.1 $g{\cdot}L^{-1}$ NAA for an hour before being inserted in the rooting medium (equal parts of Sphagnum moss and perlite by weight). The cuttings were kept in a temperature-controlled (22-23 C, 71.6-73.4 F) mist room with high humidity (mist was 14 minutes off and 6 seconds on throughout 24 hours). After 1.5 months, each cutting was classified as either rooted without calli, rooted with calli, callused without roots, or dead. There was a total of 30 cuttings.

Histology. Ten stem segments with ARs (two months after auxin treatment) were fixed in a 5:50:5 (v/v/v) formaldehyde/ethanol/acetic acid (FAA) solution overnight at room temperature. Samples were dehydrated in an ethanol series (70, 85, 95, and 100%), infiltrated with xylene, and embedded in paraffin. Then, 15 µm-thick transverse sections were cut with a rotatory microtome and stained with toluidine blue. Images were taken under a microscope (Meiji MT-51, Meiji Techno America, Campbell, CA).

Detection of endogenous hormones. The basal part (approximately 5 cm) of 10-15 cm (4-6 in) current-year semi-lignified shoots of juvenile American chestnut seedlings and poplar rooted cuttings (*Populus x euramericana*) were ground into fine powders. Approximately 100 mg of the ground sample were extracted in 1 mL ice-cold 1:1 methanol (MeOH): acetonitrile (ACN), along with 25 µL of mixed stable-isotope labeled standards. Samples were homogenized and extracted three times. After being combined and dried in a vacuum centrifuge, the extracts were then reconstituted in 100 µL 30% methanol, centrifuged to remove particulates, and then passed through a 0.8 µm polyethersulfone spin filter (Sartorius, Stonehouse, UK). Phytohormones cis-zeatin (c/tZ), dihydrozeatin (DHZ), trans-zeatin (tZR), SA, ABA, IAA, IAA- aspartate (IAA-Asp), JA, Jasmonoyl isoleucine (JA-Ile), and OPDA were quantified using a targeted multiple reaction monitoring (SRM)/isotope dilution-based liquid chromatography system with tandem mass spectrometry (LC-MS-MS) method (Gladl et al. 2020). An Ultimate 3000 (UPLC) system connected to a Thermo TSQ Altis triple quadrupole MS equipped with a heated electrospray ionization (HESI) source (Thermo Fisher Scientific, Waltham, MA) was used for the quantitative analysis. Both hormone extraction and detection were performed by the Proteomics & Mass Spectrometry Facility at the Danforth Plant Science Center (Saint Louis, MO).

To examine hormone distribution between leaf and stem tissues in current-year semi-lignified shoots of juvenile American chestnut seedlings and poplar rooted cuttings, young leaves and shoots (10-15 cm in length) (4-6 in) (without pith) were ground into fine powders in liquid N_2. Hormones were extracted from approximately 30 mg ground tissue twice using 0.5 mL of 50% acetonitrile with metal homogenization beads in a Bertin CryoLys homogenizer. After the centrifuge, the supernatant (0.9 mL) was transferred to activated and conditioned HLB cartridges for solid-phase extraction, followed by LC-MS/MS (Waters Xevo TQ-XS, Milford, MA) analysis and quantification. The internal standards (0.5 mL of 20 ppb mixture of trans-zeatin-d5 and indole-3-acetic acid-d5) were added to sample extracts. This work was performed by the Multi-User Analytical Lab (MUAL) and Metabolomic Core at Clemson University.

For the hormone study, three biological replicates were included, and a *t*-test was applied to compare American chestnut and poplar samples. The differences were considered significant at $P \leq 0.05$.

Results and Discussion

American chestnut AR can be induced with auxin. After 1.5 months with NAA treatment, an average rooting rate of 14.7%±4.9% (standard deviation, SD) was obtained for ARs without calli and 10.8% ±2.8% for ARs with calli (Fig. 1), while 70.5% (±14.5%) American chestnut cuttings formed calli only. The longest root induced in an individual cutting averaged 9.22 cm (±5.30 cm). Considering that ARs can be

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

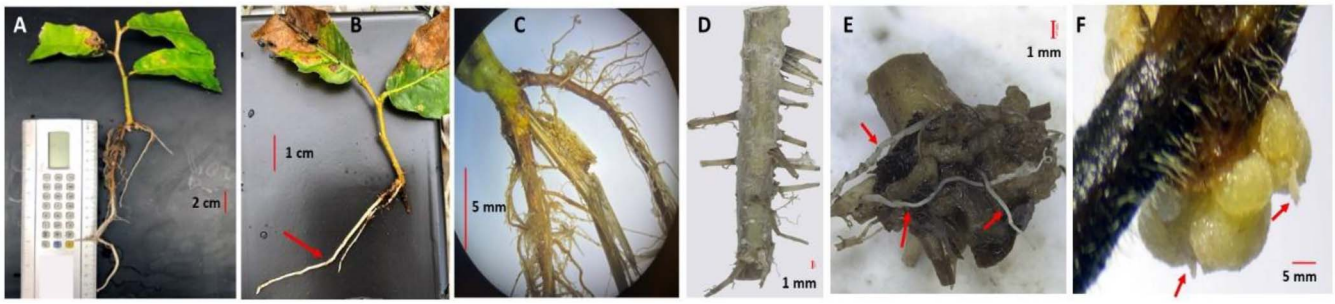

Fig. 1. Induced adventitious roots in American chestnut cuttings. A-D: roots formed directly from stem; E-F: roots emerging from callus. Cuttings were dipped into 0.1 g/L NAA for an hour before being inserted into the potting mix.

formed from calli, the rooting rate may be improved as calli can continue to differentiate, as long as leaves do not drop. In some other species, callus has been reported to develop at the base of cuttings and roots appeared from the callus (Steffens and Rasmussen 2016). None of the control cuttings (without NAA treatment) rooted or formed calli. Overall, our data corroborate the recalcitrance of American chestnut to AR induction. Galic et al. (2012) reported a rooting rate range of 46% to 65% for American chestnut juvenile softwood and semi-hardwood cuttings with 1% IBA. Different auxin types, genotypes, environmental conditions, cuttings' quality, and evaluation time might have contributed to our lower rooting rate. A higher rate of callus formation than rooting rate was also reported in *Eucalyptus* cuttings (Eliyahu et al. 2020).

In recent years, plant growth-promoting bacteria (PGPB) have gained much attention and acceptance, due to the fact that they are natural living organisms and are not pathogenic. PGPB can promote plant growth, including rooting, by producing substances such as phytohormones and preventing diseases via induced systemic resistance and inhibition of the growth of pathogens (Orozco-Mosqueda et al. 2021). Examples of PGPB are certain species of *Rhizobium* (formerly *Agrobacterium*), *Bacillus*, *Streptomyces*, *Pseudomonas*, and *Alcaligenes*. Upon infecting plants, *Rhizobium rhizogenes* (Riker, Banfield, Wright, Keitt & Sagen) Young, Kuykendall, Martinez-Romero, Kerr & Sawada integrates a portion of its transfer DNA from the root-inducing plasmid into the plant genome, resulting in the formation of "hairy roots". Morphologically, *R. rhizogenes*-induced hairy roots are very similar in structure to wild-type roots; however, hairy roots have more lateral roots and are longer, and their root systems are more branched and exhibit an agravitropic phenotype (Veena and Taylor 2007). While most available rooting reports with *R. rhizogene* are on micro-shoots, Strobel and Nachmias (1985) reported a larger root number and root mass, as well as significant increases in leaf number, stem diameter, and shoot elongation, during the first growing season after treatment of the root ball of bare-root almond trees [*Prunus dulcis* (Mill.) D.A. Webb]. In jujube (*Ziziphus jujuba* Mill.) cuttings, *R. rhizogenes* was effective in enhancing rooting percentage and root number (Hatta et al. 1995). More recent reports of *R. rhizogene* on rooting include *Prosopis alba* (Felker et al. 2005), apple rootstocks (Azmoode et al. 2017), and *Juniperus communis* L. (Khoshhal Sarmast et al. 2019). For *B. subtilis* (Ehrenberg) Cohn, a root-promotion effect was found in *Eucalyptus* (González et al 2018), mung bean [*Vigna radiata* (L.) R. Wilczek] (Hussein et al. 2016), and apple rootstocks (Karakurt et al. 2019). Considering American chestnut's high recalcitrance, it is worth exploring PGPB's effect on AR formation in American chestnut cuttings.

Direct American chestnut AR without callus formation develops a vascular system that is continuous with that of the stem. As shown in Fig. 2A-2D, AR's vascular tissues were connected with a stem's vascular system, disrupting the sclerenchyma ring of the outer phloem in the stem cutting. In contrast, the sclerenchyma ring was not distorted in the areas where calli were formed (Fig. 2G-2H), suggesting calli were derived from cortical tissue's parenchyma cells. In woody perennials, AR can arise from phloem, xylem, cambium, or even pith cells (Steffens and Rasmussen 2016). In American chestnut cuttings that formed ARs without calli, actively dividing cells (with both periclinal and anticlinal divisions) and AR meristems were found in the cambium and phloem (Fig. 2E and 2F). This is consistent with the finding of AR formation in European chestnut microshoots (Ballester et al. 1999). Our histological study did not capture imaging of rooting from callus. The origin of callus root formation and whether it shares a vascular system with the stem are unclear. In mung bean cuttings, origins of root primordia were scattered in callus cells (Park et al. 2002). According to Rasmussen and Hunt (2010), tracheids in callus differentiated and elongated to form root primordia in slash x Caribbean pine hybrids (*Pinus elliottii* var. elliottii Engelm x *P caribaea* var. hondurensis Morelet). In addition, roots were seen to differentiate from the chaotic cell organization of calli formed at the base of *Eucalyptus* cuttings, albeit at low rates (Eliyahu et al. 2020).

The endogenous hormone profile in American chestnut cuttings is not in favor of AR induction. Compared to easy-to-root poplar, levels of known AR-inhibiting hormones, i.e. CK, an inactive form of IAA amino acid conjugate aspartate (IAA-ASP), ABA, JA, JA-IlE, OPDA, and SA, were significantly higher in American chestnut cuttings (Fig. 3). In contrast, the IAA level was significantly lower in American chestnut stem tissue. Furthermore, American chestnut had a different hormone distribution profile between leaves and stems than poplar (Fig. 4). For instance, the poplar leaf and stem contained a similar level of IAA (1.2), while the ratio in American chestnut was 0.4. Also,

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

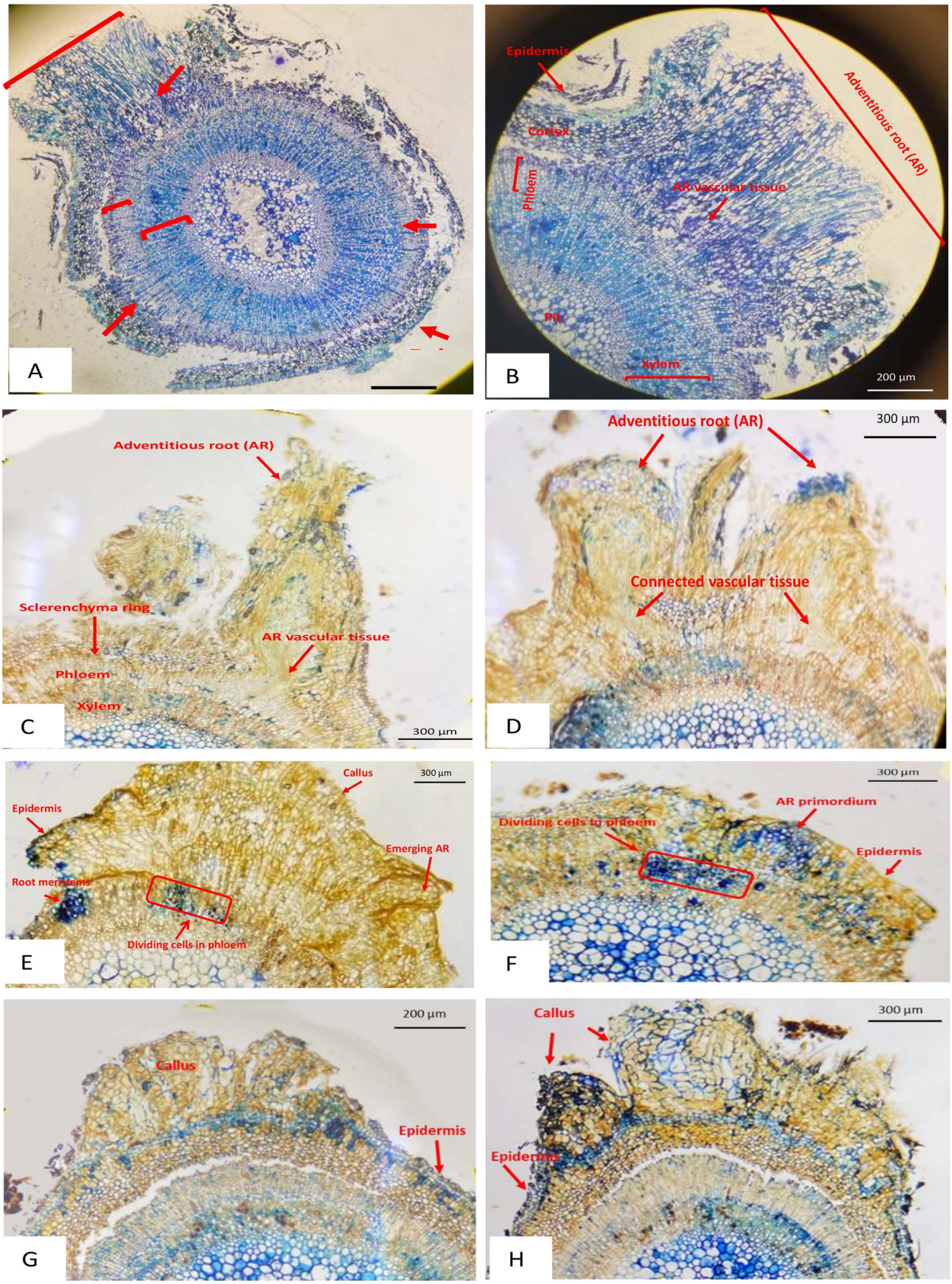

Fig. 2. **Histology of American chestnut adventitious root (AR) formation after staining with toluidine blue. A-D show induced adventitious roots with a connected vascular system with stem. E shows a root meristem and dividing cells. F indicates a root primordium and dividing cells. G-H show calli.**

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

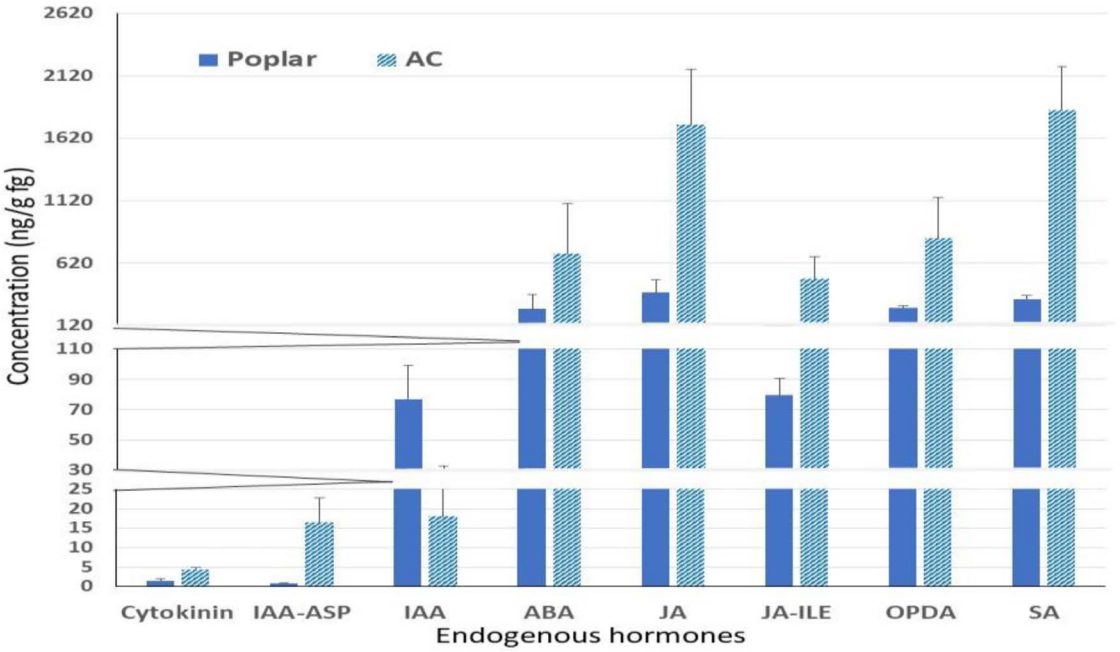

Fig. 3. Comparison of hormone levels between American chestnut and easy- to root poplar stem cuttings. Cytokinins were the combination of t-Zeatin, c-Zeatin, dihydrozeatin (DHZ), and trans-zeatin riboside (t-ZR). IAA-ASP: IAA amino acid conjugate aspartate; JA-IlE: JA- Isoleucine; SA: salicylic acid; OPDA: oxylipin 12-oxo-phytodienoic acid, a biosynthetic precursor of JA. A significant difference was found between American chestnut and poplar for all the surveyed hormones at $P<0.05$. Error bars indicate standard deviation.

American chestnut's cytokinin stem/leaf ratio was approximately 30 times higher than that in poplar.

T-zeatin, c-zeatin, DHZ, and t-ZR are biologically active forms of CKs. Neither c-zeatin nor DHZ was detectable in cuttings of poplar and American chestnut. T-zeatin was detected in American chestnut cuttings, but not in poplar. As for t-ZR, its level was doubled in American chestnut (3.36 ± 0.99 ng·g^{-1} fresh weight), when compared to the one in poplar cuttings (1.34 ± 0.53 ng·g^{-1} fresh weight). CK signaling and perception are known to be necessary for root development; however, there is a large body of evidence that indicates its negative effect on the formation of AR. For example, AR primordia development was suppressed by CK in apple cuttings (Mao et al. 2019). In *Arabidopsis thaliana* (L.) Heynh., ABA controlled root architecture by inhibiting hypocotyl adventitious root formation (Zeng et al. 2021). JA is a wound-induced hormone. Upon wounding, JA can up-regulate auxin level by increasing the amount of tryptophan, an amino acid that can be converted to form auxin, via promoting the expression of anthranilate synthase α1 (*ASA1*), a tryptophan biosynthetic gene. This may explain the important role JA plays in the regulation of AR formation in tobacco (Nicotiana tabacum L.) thin cell layers and *Arabidopsis*'s detached leaves (Fattorini et al. 2009, Gutierrez et al. 2012). However, constant long-term JA signaling is harmful to root organogenesis in *A. thaliana*, sweet potato [*Ipomoea batatas* (L.) Lam.], and apple rootstocks (Pan et al. 2021). It is suggested that the crosstalk

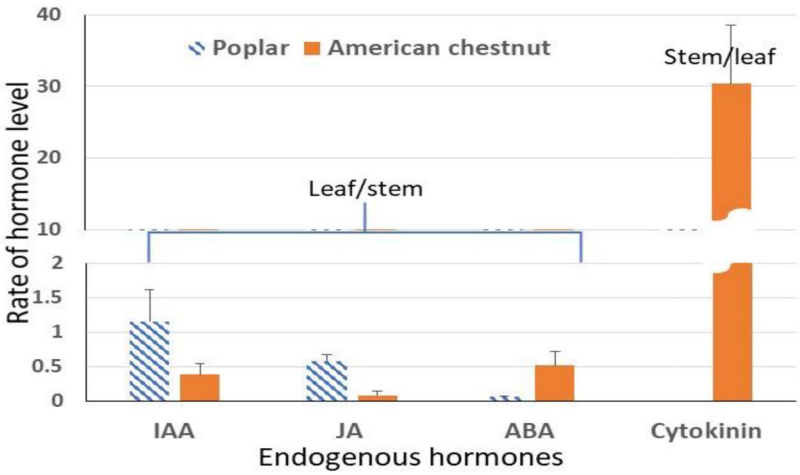

Fig. 4. Leaf/stem ratios of IAA, JA, and ABA and stem/leaf ratio of cytokinin in American chestnut and poplar. A significant difference was found between American chestnut and poplar for all the surveyed hormones at $P<0.05$. Error bars indicate standard deviation.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

between JA and CK is essential for the negative regulation of root organogenesis (Pan et al. 2021). In our study, American chestnut cuttings showed significantly higher levels of JA, JA-IlE, and OPDA (jasmonate family precursor). There are conflicting reports on the role of SA in AR formation, depending on the species. Completely different results from the current study were reported in other studies. Exogenous SA treatment promotes adventitious root formation in cucumber hypocotyls (Dong et a. 2020). SA biosynthesis in *Arabidopsis* mutants (*enhanced disease susceptibility 5*) caused fewer adventitious roots to form than roots developed in the wild type (Gutierrez et al. 2012). In contrast, SA reduced IAA-induced adventitious root numbers in apple microcuttings by enhancing IAA decarboxylation (De Klerk et al. 2011), as well as in mung bean hypocotyl cuttings (Li L 1995). In *Arabidopsis*, it was found that low concentrations of SA promoted adventitious roots and altered the architecture of root apical meristems, whereas high-concentration SA inhibited all growth processes in the root (Pasternak et al. 2019). It is suggested that low-concentration SA plays an important role in shaping root meristem structure and root system architecture (Pasternak et al. 2019). Our data indicated SA level in American chestnut cuttings was 5.6 times higher than that in poplar.

Auxin is the master regulator of AR formation and is the likely hub for the phytohormone crosstalk. The low IAA level in American chestnut cuttings (only ∼1/4 of poplar's) can be the main reason why American chestnut is notoriously recalcitrant to rooting. The auxins commonly used in AR induction include 2,4-dichloro phenoxy-acetic acid (2,4-D), IBA, IAA, and NAA. So far, IBA (Galic et al. 2012) and NAA (this study) have shown success in rooting American chestnut cuttings. It is worth testing if other auxin types have a similar effect and if there exist synergistic effects among the auxins. In European chestnut (*Castanea sativa* Mill.) microcuttings, the level of auxins, including IAA, IAA-ASP, and IBA, significantly increased one day after IBA treatment (dipping in 5 mM for 1 minute) (Ballester et al. 1999). Studies with model species indicated genes that are auxin-signaling-responsive, including *AUXIN RESPONSE FACTOR* (*ARF*) and *LATERAL ORGAN BOUNDARIES-DOMAIN* (*LOB*) gene families, and are associated with auxin transport and homeostasis, such as *YUCCA* (*YUC*) and *GRETCHEN HAGEN3* (*GH3*), are critical (Li 2021). The molecular bases for the auxin-induction of American chestnut adventitious root generation remain to be studied.

In conclusion, ARs can be induced with auxin in American chestnut cuttings, albeit the success rate is low. ARs were initiated from cambial derivatives and developed a vascular system connected with that of the stem. Compared to easy-to-root poplar, American chestnut cuttings had a low level of the root-promoting hormone IAA and a high level of other hormones that do not favor rooting induction and development. Hormone distribution between leaves and stems also differed between American chestnut and poplar. This unfavorite endogenous hormone profile may contribute to American chestnut cuttings' recalcitrance to rooting. Future studies such as metabolomics and RNA sequencing will help further understand the mechanisms of recalcitrance in American chestnut.

Literature Cited

Anagnostakis, S.L. 2012. Chestnut breeding in the United States for disease and insect resistance. Plant Dis. 96:1392–1403.

Ballester, A., M.C. San-José, N. Vidal, J.L. Fernández-Lorenzo, and A.M. Vieitez. 1999. Anatomical and biochemical events during *in vitro* rooting of microcuttings from juvenile and mature phases of chestnut. Ann. Bot. 83:619–629.

Brewer, L.G. 1995. Ecology of survival and recovery from blight in American chestnut trees (*Castanea dentata* (Marsh.) Borkh.) in Michigan. Bull. Torrey Bot. Club 122:40–57.

Clark, S.L., S.E. Schlarbaum, A.M. Saxton, and R. Baird. 2019. Eight-year blight (*Cryphonectria parasitica*) resistance of backcross-generation American chestnuts (*Castanea dentata*) planted in the southeastern United States. For. Ecol. Manag. 433:153–161.

De Klerk, G.J., H.Y. Guan, P. Huisman, and S. Marinova. 2011. Effects of phenolic compounds on adventitious root formation and oxidative decarboxylation of applied indoleacetic acid in Malus 'Jork 9'. Plant Growth. Regul. 63:175–185.

Dong, C.J., X.Y. Liu, L.L. Xie, L.L. Wang, and Q.M. Shang. 2020. Salicylic acid regulates adventitious root formation via competitive inhibition of the auxin conjugation enzyme CsGH3. 5 in cucumber hypocotyls. Planta. 252:1–5.

Eliyahu, A., Z. Duman, S. Sherf, O. Genin, Y. Cinnamon, M. Abu-Abied, R. Weinstain, A. Dag, and E. Sadot. 2020. Vegetative propagation of elite Eucalyptus clones as food source for honeybees (*Apis mellifera*); adventitious roots versus callus formation. Isr. J. Plant Sci. 67:83–97.

Fattorini, L., G. Falasca, C. Kevers, L.M. Rocca, C. Zadra, and M.M. Altamura. 2009. Adventitious rooting is enhanced by methyl jasmonate in tobacco thin cell layers. Planta. 231:155–168.

Felker, P., D. Medina, C. Soulier, G. Velicce, M. Velarde, and C. Gonzalez. 2005. A survey of environmental and biological factors (*Azospirillum spp*, *Agrobacterium rhizogenes*, *Pseudomonas aurantiaca*) for their influence in rooting cuttings of *Prosopis alba* clones. J. Arid Environ. 61:227–247.

Freinkel, S. 2007. American Chestnut. University of California Press. 284 p.

Galic, D., A. Dale, M. Alward. 2014. Vegetative propagation of American chestnut. In V International Chestnut Symposium 1019, p. 99–103.

Gradl, K., J. Taibon, N. Singh, E. Albrecht, A. Geistanger, S. Pongratz, S. Hutzler, M. Mayer, C. Kleinschmidt, C. Geletneky, V. Hofmann, D. Köppl, M. Rauh, and U. Kobold. 2020. An isotope dilution LC-MS/MS-based candidate reference method for the quantification of androstenedione in human serum and plasma. J. Mass. Spectrom. 23:16:1–10.

Gonin, M., V. Bergougnoux, T.D. Nguyen, P. Gantet, and A. Champion. 2019. What makes adventitious roots. Plants. 8:240.

Gutierrez, L., G. Mongelard, K. Flokova, D.I. Pacurar, O. Novak, P. Staswick, M. Kowalczyk, M. Pacurar, H. Demailly, G. Geiss, and C. Bellini. 2012. Auxin controls *Arabidopsis* adventitious root initiation by regulating jasmonic acid homeostasis. Plant Cell. 24:2515–2527.

Hartmann, H.T., Kester, D.E., Davies Jr., F.T., and R.L. Geneve. 2010. Plant Propagation: Principles and Practices. 8th Edition, Prentice-Hall, New Jersey, 915 p.

Hatta, M., C.A. Beyl, and S. Garton. 1995. Induction of roots on jujube softwood cuttings using *Agrobacterium rhizogenes*. HortScience 30(4):888D–888.

Keever, C. 1953, Present composition of some stands of the former oak-chestnut forest in the southern Blue Ridge Mountains. Ecology 34(1):44–54.

Khoshhal Sarmast, M., R. Kordkatoli, Z. Rezaei, and A. Ghasemnezhad. 2019. Effect of seasons, gender and *Agrobacterium rhizogenes* Strains on adventitious root induction of male and female *Juniperus communis* L. J. Ornam. Hortic. 9:23–31.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access

Li, L. 1995. Effects of resorcinol and salicylic acid on the formation of adventitious roots on hypocotyl cutting of *Vigna radiate*. J. Trop. Subtrop. Bot. 3:67–71.

Li, S.W. 2021. Molecular bases for the regulation of adventitious root generation in plants. Front. Plant Sci. 12:614072.

Mao, J., D. Zhang, Y. Meng, K. Li, H. Wang, and M. Han. 2019. Inhibition of adventitious root development in apple rootstocks by cytokinin is based on its suppression of adventitious root primordia formation. Physiol. Plant. 166:663–676.

Newhouse, A.E., L.D. Polin-McGuigan, K.A. Baier, K.E. Valletta, W.H. Rottmann, T.J. Tschaplinski, C.A. Maynard, and W.A. Powell. 2014. Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. Plant Sci. 228:88–97.

Pan, X., Z. Yang, and L. Xu. 2021. Dual roles of jasmonate in adventitious rooting. J. Exp. Bot. 72:6808–6810.

Park, J.B., K.B. Lee, and S. Lee. 2002. Histological study of callus formation and root regeneration from mung bean (*Vigna radiata* W.). J. Plant Biol. 45:170–176.

Pasternak, T., E.P. Groot, F.V. Kazantsev, W. Teale, N. Omelyanchuk, V. Kovrizhnykh, K. Palme, and V.V. Mironova. 2019. Salicylic acid affects root meristem patterning via auxin distribution in a concentration-dependent manner. Plant Physiol. 180:1725–1739.

Rasmussen, A. and M.A. Hunt. 2010. Ageing delays the cellular stages of adventitious root formation in pine. Aust. For. 73:41–46.

Steffens, B. and A. Rasmussen. 2016. The physiology of adventitious roots. Plant Physiol. 170:603–617.

Steiner, K.C., J.W. Westbrook, F.V. Hebard, L.L. Georgi, W.A. Powell, and S.F. Fitzsimmons. 2017. Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. New For. 48:317–336.

Strobel, G.A. and A.V. Nachmias. 1985. *Agrobacterium rhizogenes* promotes the initial growth of bare root stock almond. Microbiology. 131(5):1245–1249.

Veena, V. and C.G. Taylor. *Agrobacterium rhizogenes*: recent developments and promising applications. In Vitro Cell. Dev. Biol. Plant. 43:383–403.

Zeng, Y., I. Verstraeten, H.K. Trinh, T. Heugebaert, C.V. Stevens, I. Garcia-Maquilon, P.L. Rodriguez, S. Vanneste, and D. Geelen. 2021. *Arabidopsis* hypocotyl adventitious root formation is suppressed by ABA signaling. Genes. 12:1141.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-19 via free access