Agronomía Colombiana
ISSN: 0120-9965
ISSN: 2357-3732
Universidad Nacional de Colombia, Facultad de Agronomía

Maggioni, Renata de Almeida; Latoh, Leandro Porto; Vieira, Leandro Marcolino; Netto, Emilio Romanini; Zuffellato-Ribas, Katia Christina

Ginkgo biloba L. mini-cuttings: indole butyric acid, substrates, and biochemical composition of the mother plants

Agronomía Colombiana, vol. 38, no. 3, 2020, September-December, pp. 429-435
Universidad Nacional de Colombia, Facultad de Agronomía

DOI: https://doi.org/10.15446/agron.colomb.v38n3.86430

Ginkgo biloba L. mini-cuttings: indole butyric acid, substrates, and biochemical composition of the mother plants

Miniestacas de *Ginkgo biloba* L.: ácido indolbutírico, substratos y composición bioquímica de las plantas madre

Renata de Almeida Maggioni[1], Leandro Porto Latoh[1], Leandro Marcolino Vieira[1]*, Emilio Romanini Netto[2], and Katia Christina Zuffellato-Ribas[1]

ABSTRACT

The objective of this study was to evaluate the viability of the *Ginkgo biloba* mini-cutting technique, as well as the influence of substrates and different concentrations of indole butyric acid (IBA) on adventitious rooting in addition to the protein and sugar content in the mini-cutting. Mini-cuttings were 4 ± 1 cm in length, with the bases immersed in solutions of 0, 1000, 2000, and 3000 mg L^{-1} IBA. They were then planted in polypropylene tubes using two substrates (vermiculite and Tropstrato®) and maintained under greenhouse conditions for 60 d. The experiment was carried out with a 2 × 4 factorial scheme (substrates × IBA). There was no influence of IBA application on the promotion of rhizogenesis in *Ginkgo biloba* mini-cuttings. The rooting percentages were higher than 55% regardless of the treatment used. The vermiculite substrate showed a higher number of roots (4.94) and lower mortality (11.60) of mini-cuttings than Tropstrato®. We conclude that the mini-cutting technique is feasible for *Ginkgo biloba*, and the use of IBA is not necessary. We found that the induction of adventitious rooting depended on the biochemical composition of the mother plants, due to the translocation of non-reducing sugars and leaf proteins for root formation.

Key words: vegetative propagation, medicinal plants, plant growth regulators, medicinal plant cloning, rooting.

RESUMEN

El objetivo de este estudio fue evaluar la viabilidad de la técnica de miniestacas de *Ginkgo biloba*, así como la influencia de diferentes sustratos y diferentes concentraciones de ácido indolbutírico (IBA) en la formación de raíces adventicias además del contenido de proteínas y azúcares en las miniestacas. Las miniestacas tenían 4 ± 1 cm de longitud, y sus bases se sumergieron en soluciones de 0, 1000, 2000 y 3000 mg L^{-1} IBA. Luego las miniestacas se plantaron en tubos de polipropileno usando dos sustratos (vermiculita y Tropstrato®), bajo condiciones de invernadero durante 60 d. El experimento se llevó a cabo en un esquema factorial 2 × 4 (sustratos × IBA). No hubo influencia de la aplicación de IBA en la promoción de la rizogénesis de las miniestacas de *Ginkgo biloba*. Los porcentajes de enraizamiento fueron superiores al 55%, independientemente del tratamiento utilizado. El sustrato de vermiculita mostró un mayor número de raíces (4.94) y menor mortalidad (11.60) de miniestacas que el Tropstrato®. Por lo tanto, se puede concluir que la técnica de miniestaca es factible para *Ginkgo biloba*, y que el uso de IBA no es necesario. Se encontró que la inducción de raíces adventicias depende de la composición bioquímica de las plantas madre, debido a la translocación de azúcares no reductores y proteínas de la hoja para la formación de las raíces.

Palabras clave: propagación vegetativa, plantas medicinales, reguladores del crecimiento, clonación de plantas medicinales, enraizamiento.

Introduction

Ginkgo biloba L. (Ginkgoaceae) is a deciduous species from Asia whose extracts, leaves, and fruits have been used in the treatment of mental illnesses for more than 2000 years (Zhang *et al.*, 2011). It is one of the most studied and popular medicinal plants with contents of gincolids and flavonoids, free radical scavenging properties, and a proven effect on the human cardiovascular system, particularly in cerebral circulation (Lin *et al.*, 2008; Van Beek & Montoro, 2009; Song *et al.*, 2010; El-Ghazaly *et al.*, 2015). Besides its medicinal potential, gingko also has a high ornamental potential, with fan-shaped leaves, lush color that turns greenish-brown in autumn and yellowish in winter (Tommasi & Scaramuzzi, 2004; Bitencourt *et al.*, 2010).

Because it is a dioecious species, seedling production requires plants of both sexes, and this hinders species

Received for publication: 15 May, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.86430

[1] Programa de Pós-graduação em Agronomia (Produção Vegetal), Universidade Federal do Paraná (UFPR), Curitiba, Parana (Brazil).
[2] Programa de Pós-graduação em Meio Ambiente e Desenvolvimento, Universidade Federal do Paraná (UFPR), Curitiba, Parana (Brazil).
* Corresponding author: leandro.marcolino@ufpr.br

propagation (Singh *et al.*, 2008). In addition, vegetative propagation of the species requires plant growth regulator application and, even so, the produced plants have low vigor of (Acharya *et al.*, 2001; Valmorbida & Lessa, 2008; Bitencourt *et al.*, 2010). Based on this, it is necessary to develop and evaluate vegetative rescue techniques to optimize asexual propagation.

Vegetative propagation of plants has been one of the most important methods of clonal forestry (Martins *et al.*, 2011) in recent decades, representing significant gains for the industry and society (Steffens & Rasmussen, 2016) and ensuring uniformity in planting, higher productivity, and low cost and allowing the selection of desirable characteristics (Xavier *et al.*, 2009; Wendling *et al.*, 2016). Mini-cutting is a vegetative propagation technique that can be considered a specialization of conventional cutting. This technique basically consists of the use of seedlings propagated by seeds or rooted cuttings that will constitute a mini garden (Alfenas *et al.*, 2009). The mini-cutting success is mainly related to cell and tissue rejuvenation that favors meristem differentiation to rooting formation (Ferriani *et al.*, 2010).

The rooting and survival of mini-cuttings can be influenced by several factors such as physiological conditions, type and position of propagules, collection season, juvenility, substrate, and the use of plant growth regulators for rooting induction (Carpanezzi *et al.*, 2001; Rezende, 2007). The level of endogenous auxin is considered a critical factor in adventitious rooting in many tree species, requiring the use of exogenous auxins to induce rooting (Wendling *et al.*, 2015).

The objective of this study was to evaluate the viability of the *Ginkgo biloba* mini-cutting technique, testing different substrates and concentrations of indole butyric acid (IBA). In addition, the contents of sugars and proteins related to the rhizogenesis of the species will verify the relationship between adventitious rooting and the biochemical composition of cuttings.

Materials and methods

Experiment I - *Gingko biloba* mini-cuttings

Gingko biloba shoots were collected on October 16, 2017 from ~30 mini-stumps conducted in a mini-garden system (Fig. 1A) at the Forest species propagation laboratory (Embrapa Forests, Colombo-PR, 25°19' S and 49°09' W, 950 m a.s.l.). After collection, the plant material was kept in styrofoam boxes with water to avoid dehydration. The material was then transported to the Macropropagation Laboratory at the research and study group on cuttings (GEPE), located at the Federal University of Paraná (UFPR), in Curitiba - PR (25°44' S and 49°23' W, 920 m a.s.l.). From the shoots of the mini-stumps, stem mini-cuttings were obtained with 4 ± 1 cm length, with a bevel cut in the base and straight cut at the tip, maintaining a leaf in the apical portion (Fig. 1B).

After preparation, mini-cutting bases were immersed in the indole butyric acid (IBA) plant growth regulator in

FIGURE 1. A) Mini-stumps, B) mini-cutting, and C) experiment installed in the greenhouse.

50% hydroalcoholic solution for 10 sec (Bitencourt *et al.*, 2010), according to the treatments 0 mg L^{-1}, 1000 mg L^{-1}, 2000 mg L^{-1} and 3000 mg L^{-1}. The planting was carried out in polypropylene tubes with 53 cm^3 capacity, using two substrates separately: vermiculite of fine granulometry and the commercial substrate Tropstrato®. The cuttings were planted at about ⅓ the depth of the base and maintained for 60 d in a greenhouse with intermittent mist (temperature of 24°C ± 2°C and 80% relative humidity) (Fig. 1C), located in the Biological Sciences Department of the Federal University of Paraná.

The experiment was arranged in a completely randomized design with a 2 × 4 factorial scheme (substrates × IBA concentrations) for a total of 8 treatments with 4 replicates and 14 mini-cuttings per experimental unit, totaling 448 mini-cuttings.

After 60 d, the following variables were evaluated: percentage of rooted mini-cuttings (mini-cuttings that emitted roots of at least 1 mm in length) (Fig. 2A), number of roots per mini-cutting, average length of three major roots per mini-cutting (cm), percentage of mini-cuttings with calluses (mini-cuttings alive, without roots, with formation of undifferentiated cell mass in the base) (Fig. 2B), percentage of live mini-cuttings (mini-cutting without calluses and without roots) (Fig. 2C), percentage of dead mini-cuttings (mini-cuttings with necrotic tissues); percentage of mini-cutting shoots (live mini-cuttings with or without roots and callus, showing shoots of new leaves) (Fig. 2A), and percentage of mini-cuttings that retained their original leaves (live mini-cuttings, with or without roots and callus, which retained the original leaves at the time of evaluation) (Fig. 2A).

Experiment II - Biochemical composition of *Gingko biloba* mini-cuttings

For the biochemical analysis, ten *Ginkgo biloba* mini-cuttings were used at the time of the rooting and ten mini-cuttings at the time of evaluation at 60 d. For the quantification of the total protein content, the method described by Bradford (1976) was used and the total soluble sugar contents were determined by the phenol-sulfuric method described by Dubois *et al.* (1956).

Statistical analysis

For both experiments, the treatment variances were tested for homogeneity using the Bartlett's test (P<0.05) and normality test [Shapiro-Wilk (W)]. Subsequently, the variables were subjected to analysis of variance (ANOVA) and those with significant differences in relation to the F test had their means compared with the Tukey test (P<0.01 and P<0.05). Only the variables live mini-cuttings, percentage of mortality, callus formation, and percentage of mini-cuttings that retained their original leaves did not show normality but were homogeneous with the Bartlett test.

Results and discussion

Experiment I - *Gingko biloba* mini-cuttings

There was no significant interaction (P<0.05) between the substrate × IBA concentrations for all the evaluated

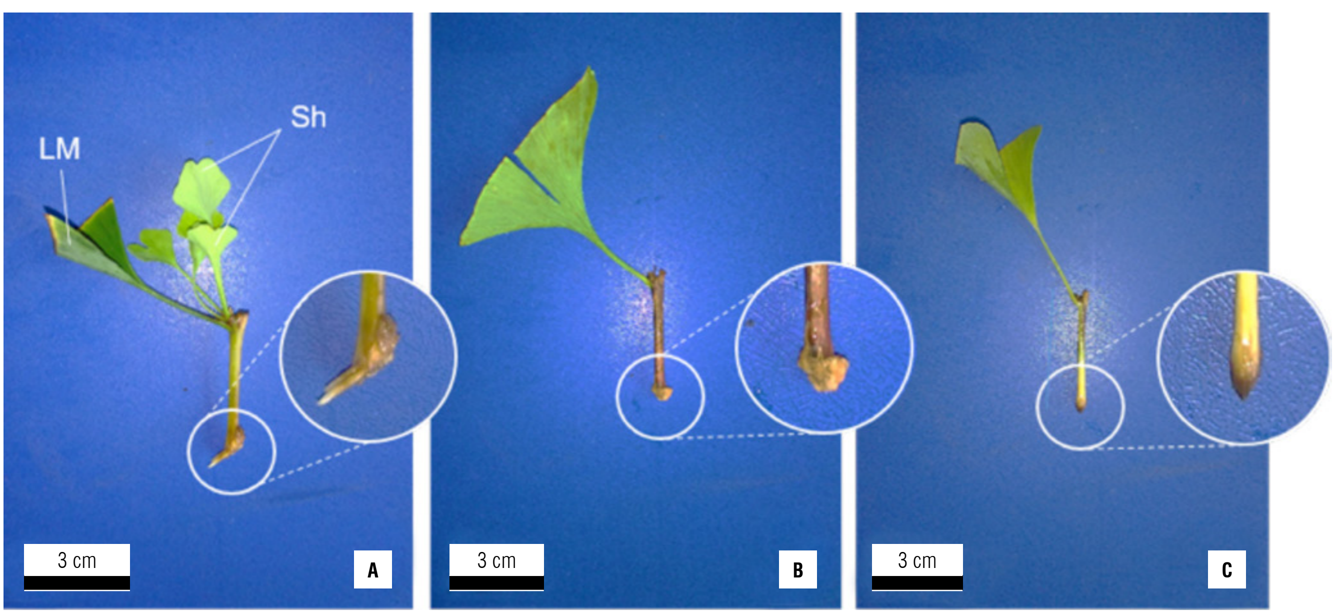

FIGURE 2. A) Rooted mini-cutting, B) mini-cutting with callus and C) live mini-cutting. LM - leaf maintenance; Sh - shoots of new leaves.

variables, indicating that these factors can act alone in the adventitious rooting of the species. The concentrations of IBA did not influence the induction and development of *Ginkgo biloba* roots (Tab. 1). In a previous conventional cutting study of the species, Valmorbida and Lessa (2008) observed that the IBA influenced adventitious rooting formation, when compared to the treatment without the use of this plant growth regulator, with rooting percentage results up to 80.55%. Bitencourt *et al.* (2010) found statistical differences with higher concentrations of IBA (4000 and 8000 mg L^{-1}) bound in talc on ginkgo cutting rooting (45.00 and 46.25% rooting percentages, respectively).

Thus, since the effect of IBA on the rooting of the species has not been observed, the results obtained in this study are promising considering the high rooting rates (mean of 62.05%). This result shows a positive effect of the rejuvenation of the plant material in the adventitious rooting of *Ginkgo biloba* and indicates the potential of the mini-cutting technique for the species, resulting in a reduction in the cost of production since plant regulators are not used for root induction.

One of the advantages of the mini-cutting technique is precisely the reduced need for exogenous auxin application due to the reinvigoration of parent plants (Stuepp *et al.*, 2015; Stuepp *et al.*, 2017b). Similarly, Stuepp *et al.* (2017a) confirm the efficiency of the mini-cutting technique for the species, resulting in a high rooting index (92.5%). The low percentages of live and dead mini-cuttings in all treatments are a consequence of the high levels of rooting with fast formation, demonstrating the adaptation of the material to greenhouse conditions. The high potential of rhizogenesis of *Ginkgo biloba* mini-cuttings validates the efficiency of the mini-cutting technique for the species.

Regarding the variable mini-cuttings with callus, the Tropstrato® substrate promoted a higher percentage of callus that differed statistically from the vermiculite substrate. The treatment without IBA application and with 1000 mg L^{-1} showed a higher percentage of callus when compared to the others; however, these treatments did not have an effect on the rooting of cuttings. The treatments that showed the highest percentage of calluses may be promising, indicating that a longer permanence of the mini-cuttings in the greenhouse could result in root induction. This fact is justified since the treatments with higher percentages of calluses were the same with lower percentages of rooting (Tab. 1). This inverse relationship between the percentage of callus and adventitious root formation has already been reported by other authors who observed that the formation of adventitious roots in *Ginkgo biloba* can occur from the callus tissue formed at the base of cuttings or mini-cuttings. This suggests that root induction could result from a longer permanence of mini-cuttings in the greenhouse (Valmorbida & Lessa, 2008; Bitencourt *et al.*, 2010; Stuepp *et al.*, 2017a).

There was a significant difference between the concentrations of IBA in the percentage of shoots, with the highest percentages observed in the treatments without IBA application and in the concentration of 3000 mg L^{-1} (Tab. 1). According to Moubayidin *et al.* (2010), root growth occurs when, in the apical meristem, cell division prevails over differentiation. This occurs when there is a greater concentration of auxins promoting cell division, in contrast to the cytokinin concentrations that promote cellular differentiation.

The auxin:cytokinin ratio regulates tissue morphogenesis. While a high auxin:cytokinin ratio stimulates

TABLE 1. Rooting percentage (R), number of roots per mini-cutting (NR), average length of three major roots per mini-cutting (LR), live mini-cuttings (A), percentage of mortality (M), callus formation (C), percentage of mini-cutting shoots (Sh), percentage of mini-cuttings that retained their original leaves (LM), subjected to substrates and concentrations of indole butyric acid (IBA) in *Ginkgo biloba* mini-cuttings.

Substrates	R (%)	NR	LR (cm)	A (%)	M (%)	C (%)	Sh (%)	LM (%)
Vermiculite	68.30 ^{a}	4.94 ^{a}	1.04 ^{a}	13.80 ^{a}	11.60 ^{b}	6.68 ^{b}	33.92 ^{a}	84.37 ^{a}
Tropstrato®	55.80 ^{a}	3.73 ^{b}	1.02 ^{a}	12.50 ^{a}	21.42 ^{a}	11.27 ^{a}	29.91 ^{a}	74.10 ^{a}
IBA								
0 mg L^{-1}	58.03 ^{a}	3.78 ^{a}	1.12 ^{a}	16.96 ^{a}	15.17 ^{b}	12.71 ^{a}	42.85 ^{a}	80.35 ^{a}
1000 mg L^{-1}	59.82 ^{a}	4.84 ^{a}	1.11 ^{a}	6.25 ^{b}	24.10 ^{a}	9.82 ^{ab}	24.99 ^{b}	74.10 ^{a}
2000 mg L^{-1}	60.71 ^{a}	4.56 ^{a}	0.97 ^{a}	16.90 ^{a}	14.28 ^{b}	8.03 ^{b}	26.78 ^{b}	83.03 ^{a}
3000 mg L^{-1}	69.64 ^{a}	4.15 ^{a}	0.93 ^{a}	12.50 ^{ab}	12.49 ^{b}	5.35 ^{b}	33.03 ^{ab}	79.46 ^{a}
CV(%)	31.70	29.75	42.93	53.02	32.92	37.72	34.58	17.96

Means followed by the same letter in each variable do not differ according to the Tukey test at 5% probability. CV: coefficient of variation.

the formation of adventitious roots, a low ratio leads to higher growth of the shoot (Taiz *et al*., 2017). According to Agulló-Antón *et al.* (2011) and Agulló-Antón *et al.* (2014), exogenous auxin supplementation in cuttings affect the endogenous cytokinin concentration, influencing adventitious rooting. Souza and Miranda (2006) found in an *in vitro* study that the proliferation of shoots and root formation in *Gerbera jamesonni* depends on the balance between auxin and cytokinin. Although a greater number of shoots was observed in the treatment without synthetic auxin application, adventitious rooting was not influenced, indicating that the presence of leaves induced an auxin-favorable balance.

It is possible to verify a close relationship between the rooting percentage and adventitious root development by maintaining mini-cutting leaves in all treatments (Tab. 1). The importance of leaf maintenance for these two variables has been reported in the literature, especially related to the presence of certain leaf compounds, such as carbohydrates, auxins and rooting cofactors, which can be translocated by phloem to the base of the cuttings, thus stimulating root formation (Bona & Biasi, 2010; Fragoso *et al*., 2015).

Experiment II - Biochemical composition of *Gingko biloba* mini-cuttings

Adventitious rooting is influenced by the biochemical composition of propagules (Yan *et al*., 2017). Some studies have demonstrated the correlation between rooting and carbohydrate content (Aslmoshtaghi & Reza-Shahsavar, 2010; Ragonezi *et al*., 2010; Denaxa, *et al.,* 2012).

The carbohydrates translocated by phloem are non-reducing sugars such as sucrose, because they are less reactive than reducing sugars (Taiz *et al*., 2017). This fact can be seen in Figure 3, where it is possible to verify a decrease in the non-reducing sugar concentration at the time of mini-cutting evaluation (60 d after experimental set up). Additionally, it is possible to observe a high level of non-reducing sugars at the moment of root evaluation.

These carbohydrates translocated by phloem can be used as an energetic resource in root induction and development (Aslmoshtaghi & Reza-Shahsavar, 2010; Souza *et al*., 2015) and as a carbon source for biosynthesis of amino acids and nucleic acids (Fachinello *et al*., 2005). They can also act in the regulation of gene expression (Wang & Ruan, 2013). Thus, the reduction of endogenous carbohydrate levels indicates that these sugars were used during root emission and growth (Husen & Pal, 2007), as demonstrated in the present study.

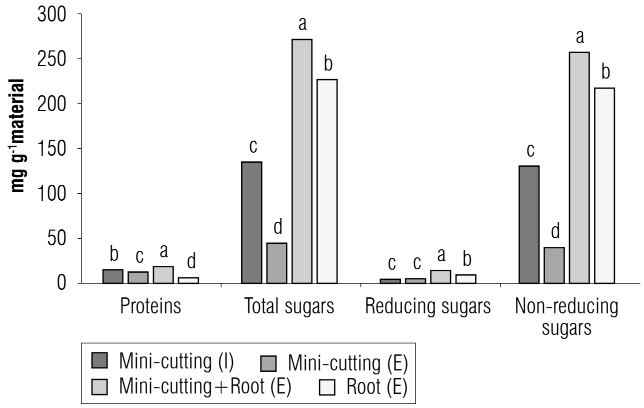

FIGURE 3. Biochemical composition of different organs of *Ginkgo biloba* and collection time. I - Installation; E - evaluation. Means followed by the same letter do not differ according to the Tukey test ($P<0.05$).

The quantity of protein in the mini-cuttings observed at the moment of evaluation was also lower than that observed at the moment of setting up the experiment (Fig. 3). Proteins are biochemical compounds associated with rooting, that can help in the induction, formation and development of adventitious roots (Taiz *et al*., 2017), and may be involved in the signaling and biosynthesis of auxins (Franklin *et al.,* 2011; Hornitschek *et al.,* 2012; Zhang *et al*., 2017).

When evaluated separately from cuttings, the reduction in the levels of non-reducing sugars and proteins during rooting that is associated with the high contents of these compounds in the roots (Fig. 3), indicates an ideal redirection of the source of drain energy. This fact reassures the importance of the nutritional status of the stock plant since it is directly related to the formation of the root system (Hartmann *et al*., 2011), as confirmed by the high rooting indexes in the present study. Furthermore, these results reinforce the importance of the nutritional status of the stock plant that directly affects the mini-cutting rooting and survival, and that is justified by the high rooting results observed in the present study.

Conclusion

The mini-cutting technique is feasible for the production of *Ginkgo biloba* seedlings regardless of the substrate used. Additionally, we concluded it is not necessary to use a plant growth regulator to induce rooting.

The induction of adventitious roots is dependent on the biochemical composition of the mother plants.

Acknowledgments

The authors would like to acknowledge the GEPE (Research and Study Group on Cuttings) and UFPR (Federal

University of Paraná) for providing the infrastructure used in this research and the Coordination for the Improvement of Higher Education Personnel (CAPES), by financial support of the research and scholarships granted.

Author's contributions

RDAM, LPL, LMV, and KCZ formulated the research goals and aims, RDAM, LPL, LMV, and ERN developed the methodology, RDAM, LMV, and KCZ conducted the research and investigation process, RDAM prepared the initial draft and created the visualization/data presentation, LPL implemented the software, LPL and LMV applied the statistical techniques to analyze study data, ERN and KCZ carried out the critical review, commentary, and revision of the manuscript, KCZ verified the overall replication/reproducibility of the results/experiments, provided the study materials, oversaw, managed and coordinated the research activity planning and execution, and acquired the financial support for the project.

Literature cited

Acharya, M., Ghosh, T. C., Acharya, R., & Acharya, K. (2001). *Ginkgo* propagation by simple cutting. *Indian Forester*, *127*(7), 827–828.

Agulló-Antón, M. Á., Sánchez-Bravo, J., Acosta, M., & Druege, U. (2011). Auxins or sugars: what makes the difference in the adventitious rooting of stored carnation cuttings? *Journal of Plant Growth Regulation*, *30*(1), 100–113. https://doi.org/10.1007/s00344-010-9174-8

Agulló-Antón, M. Á., Ferrández-Ayela, A., Fernández-García, N., Nicolás, C., Albacete, A., Pérez-Alfocea, F., Sánchez-Bravo, J., Pérez-Pérez, J. M., & Acosta, M. (2014). Early steps of adventitious rooting: morphology, hormonal profiling and carbohydrate turnover in carnation stem cuttings. *Physiologia Plantarum, 150*(3), 446–462. https://doi.org/10.1111/ppl.12114

Alfenas, A. C., Zauza, E. A. V., Mafia, R. G., & Assis, T. F. (2009). *Clonagem e doenças do eucalipto* (2nd ed.). Editora UFV.

Aslmoshtaghi, E., & Reza-Shahsavar, A. (2010). Endogenous soluble sugars, starch contents and phenolic compounds in easy- and difficult-to-root olive cuttings. *Journal of Biological and Environmental Sciences, 4*(11), 83–86.

Bitencourt, J., Zuffellato-Ribas, K. C., & Koehler, H. S. (2010). Estaquia de *Ginkgo biloba* L. utilizando três substratos. *Revista Brasileira de Plantas Medicinais*, *12*(2), 135–140. https://doi.org/10.1590/S1516-05722010000200002

Bona, C. M., & Biasi, L. A. (2010). Influence of leaf retention on cutting propagation of *Lavandula dentata* L. *Revista Ceres*, *57*(4), 526–529. https://doi.org/10.1590/S0034-737X2010000400014

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Carpanezzi, A. A., Tavares, F. R., & Sousa, V. A. (2001). Estaquia de Corticeira-do-Banhado (*Erythrina crista-galli* L.). *Embrapa Florestas-Comunicado Técnico 64*.

Denaxa, N. K., Vemmos, S. N., & Roussos, P. A. (2012). The role of endogenous carbohydrates and seasonal variation in rooting ability of cuttings of an easy and a hard to root olive cultivars (*Olea europaea* L.). *Scientia Horticulturae, 143*, 19–28. https://doi.org/10.1016/j.scienta.2012.05.026

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analitycal Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

El-Ghazaly, M. A., Sadik, N. A., Rashed, E. R., & Abd-El-Fattah, A. A. (2015). Neuroprotective effect of EGb761® and low-dose whole-body γ-irradiation in a rat model of Parkinson's disease. *Toxicology and Industrial Health*, *31*(12), 1128–1143. https://doi.org/10.1177/0748233713487251

Fachinello, J. C., Hoffmann, A., & Nachtigal, J. C. (2005). *Propagação de plantas frutíferas*. EMBRAPA informação tecnológica.

Ferriani, A. P., Zuffellato-Ribas, K. C., & Wendling, I. (2010). Miniestaquia aplicada a espécies florestais. *Revista Agroambiente On-line, 4*(2), 102–109. https://doi.org/10.18227/1982-8470ragro.v4i2.363

Fragoso, R. O., Witt, N. G. P. M., Obrzut, V. V., Valério, S., Zuffellato-Ribas, K. C., & Stuepp, C. A. (2015). Maintenance of leaves and indole butyric acid in rooting of juvenile Japanese Flowering Cherry cuttings. *Revista Brasileira de Ciências Agrárias, 10*(1), 97–101. https://doi.org/10.5039/agraria.v10i1a5111

Franklin, K. A., Lee, S. H., Patel, D., Kumar, V., Spartz, A. K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J. D., Wigge, P. A., & Gray, W. M. (2011). Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proceedings of the National Academy of Sciences*, *108*(50), 20231–20235. https://doi.org/ 10.1073/pnas.1110682108

Hartmann, H. T., Kester, D. E., Davies Jr, F. T., & Geneve, R. L. (2011). *Hartmann and Kester's plant propagation: principles and practices* (8th ed.). Prentice Hall.

Hornitschek, P., Kohnen, M. V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J. M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I., & Fankhauser, C. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *The Plant Journal*, *71*(5), 699–711. https://doi.org/10.1111/j.1365-313X.2012.05033.x

Husen, A., & Pal, M. (2007). Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. *New Forests, 34*(3), 223–233. Doi: https://doi.org/10.1007/s11056-007-9050-y

Lin, L. Z., Chen, P., Ozcan, M., & Harnly, J. M. (2008). Chromatographic profiles and identification of new phenolic components of *Ginkgo biloba* leaves and selected products. *Journal of Agricultural and Food Chemistry, 56*(15), 6671–6679. https://doi.org/10.1021/jf800488x

Martins, F. B., Soares, C. P. B., Leite, H. G., Souza, A. L., & Castro, R. V. (2011). Índices de competição em árvores individuais de eucalipto. *Pesquisa Agropecuária Brasileira, 46*(9), 1089–1098. https://doi.org/10.1590/S0100-204X2011000900017

Moubayidin, L., Perilli, S., Ioio, R. D., Mambro, R. D. M., Costantino, P., & Sabatini, S. (2010). The rate of cell differentiation controls the *Arabidopsis* root meristem growth phase. *Current Biology, 20*(12), 1138–1143. https://doi.org/10.1016/j.cub.2010.05.035

Ragonezi, C., Klimaszewska, K., Castro, M. R., Lima, M., Oliveira, P., & Zavattieri, M. A. (2010). Adventitious rooting of conifers: influence of physical and chemical factors. *Trees, 24*(6), 975–992. https://doi.org/10.1007/s00468-010-0488-8

Rezende, A. A. (2007). *Enraizamanto de estacas de candeia (Eremanthus erythropappus (DC.) MacLeish).* [Master's thesis, Universidade Federal de Lavras]. UFLA Repository. http://repositorio.ufla.br/bitstream/1/2714/1/DISSERTA%C3%87%C3%83O_Enraizamento%20de%20estacas%20de%20candeia%20(Eremanthus%20erytropappus%20DC.)%20Mac%20Leish.pdf

Singh, B., Kaur, P., Gopichand, Singh, R. D., & Ahuja, P. S. (2008). Biology and chemistry of *Ginkgo biloba*. *Fitoterapia, 79*(6), 401–418. https://doi.org/10.1016/j.fitote.2008.05.007

Song, J., Fang, G., Zhang, Y., Deng, Q., & Wang, S. (2010). Fingerprint analysis of *Ginkgo biloba* leaves and related health foods by high-performance liquid chromatography/electrospray ionization-mass spectrometry. *Journal of AOAC International, 93*(6), 1798–1805.

Souza, C. M., & Miranda, R. M. (2006). Otimização do balanço entre auxina e citocinina para multiplicação *in vitro* de *Gerbera jamesonni* var. 'Ornela'. *Revista Agronomia*, *40*(1–2), 66–72.

Souza, E. R., Lenk, F. L., Ono, E. O., & Rodrigues, J. D. (2015). Conteúdo de carboidratos em estacas de videira do porta-enxerto cv. IAC 572. *Brazilian Journal of Applied Technology for Agricultural Science, 8*(2), 7–15. https://doi.org/10.5935/PAeT.V8.N2.01

Steffens, B., & Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology*, 170, 603–617. https://doi.org/10.1104/pp.15.01360

Stuepp, C. A., Zuffellato-Ribas, K. C., Koehler, H. S., & Wendling, I. (2015). Rooting mini-cuttings of *Paulownia fortunei* var. *Mikado* derived from clonal mini-garden. *Revista Árvore, 39*(3), 497–504. https://doi.org/10.1590/0100-67622015000300010

Stuepp, C. A., Fragoso, R. O., Maggioni, R. A., Zuffellato-Ribas, K. C., & Wendling, I. (2017a). Vegetative rescue and *ex vitro* plants production system for *Ginkgo biloba* by cuttings and mini-cuttings. *Revista Brasileira de Plantas Medicinais*, *19*(2), 300–303.

Stuepp, C. A., Wendling, I., Koehler, H. S., & Zuffellato-Ribas, K. C. (2017b). Successive mini-cuttings collection in *Piptocarpha angustifolia* mini-stumps: Effects on maturation, adventitious root induction and root vigor. *Acta Scientiarum Agronomy, 39*(2), 245–253. https://doi.org/10.4025/actasciagron.v39i2.31059

Taiz, L., Zeiger, E., Møller, I. A., & Murphy, A. (2017). *Fisiologia e Desenvolvimento Vegetal* (6th ed.). Artmed.

Tommasi, F., & Scaramuzzi, F. (2004). *In vitro* propagation of *Ginkgo biloba* by using various bud cultures. *Biologia Plantarum*, *48*, 297–300. https://doi.org/10.1023/B:BIOP.0000033460.75432.d1

Valmorbida, J., & Lessa, A. O. (2008). Enraizamento de estacas de *Ginkgo biloba* tratadas com ácido indolbutírico e ácido bórico. *Ciência e agrotecnologia, 32*(2), 398–401. https://doi.org/10.1590/S1413-70542008000200008

Van Beek, T. A., & Montoro, P. (2009). Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. *Journal of Chromatography*, *1216*(11), 2002–2032. https://doi.org/10.1016/j.chroma.2009.01.013

Wang, L., & Ruan, Y. (2013). Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Science*, *4*, 1–9. https://doi.org/10.3389/fpls.2013.00163

Wendling, I., Brooks, P. R., & Trueman, S. J. (2015). Topophysis in *Corymbia torelliana* × *C. citriodora* seedlings: adventitious rooting capacity, stem anatomy, and auxin and abscisic acid concentrations. *New Forests*, *46*, 107–120. https://doi.org/10.1007/s11056-014-9451-7

Wendling, I., Stuepp, C. A., & Zuffellato-Ribas, K. C. (2016). Araucaria clonal forestry: types of cuttings and mother tree sex in field survival and growth. *Cerne*, *22*(1), 19–26. https://doi.org/10.1590/01047760201622012105

Xavier, A., Wendling, I., & Silva, R. L. (2009). *Silvicultura clonal: princípios e técnicas* (2nd ed.). Editora UFV.

Yan, S. P., Yang, R. H., Wang, F., Sun, L. N., & Song, X. S. (2017). Effect of auxins and associated metabolic changes on cuttings of hybrid aspen. *Forests*, *8*(4), Article 117. https://doi.org/10.3390/f8040117.

Zhang, L., Lam, W. P., Lü, L., Wang, Y. X., Wong, Y. W., Lam, L. H., Tang, H. C., Wai, M. S., Mak, Y. T., Wang, M., & Yew, D. T. (2011). How would composite traditional chinese medicine protect the brain - an example of the composite formula "Pien Tze Huang". *Current Medicinal Chemistry*, *18*(23), 3590–3594. https://doi.org/10.2174/092986711796642535

Zhang, W., Fan, J., Tan, Q., Zhao, M., & Cao, F. (2017). Mechanisms underlying the regulation of root formation in *Malus hupehensis* stem cuttings by using exogenous hormones. *Journal of Plant Growth Regulation, 36*(1), 174–185. https://doi.org/10.1007/s00344-016-9628-8