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SHORT COMMUNICATION

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A new and rapid micropropagation protocol for *Eucalyptus grandis* Hill ex Maiden

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Abstract

Aim of the study: We developed a faster micropropagation protocol specifically designed for Eucalyptus grandis. Eucalyptus breeding programs use micropropagation protocols to obtain high quality cloned seedlings, but current protocols are excessively time consuming.

Area of the study: The protocol has been developed in Argentina, but it can be applied in anywhere.

Materials and methods: We used nodal segments as initial explants to obtain micropropagated shoots, which were then simultaneously rooted ex vitro and acclimated in a hydroponic system. Nodal segments were cultured in a MS medium supplemented with 1 mg l-1 6-benzylaminopurine, 30 g l-1 sucrose, 1 g l-1 active charcoal and 8 g l-1 agar and incubated for four weeks at 25 ± 2°C under 16 h day photoperiod. Then, micropropagated shoots were exposed 15 seconds to 5000 ppm indol-butyric acid prior to being transferred to a hydroponic system, allowing simultaneous ex vitro rooting and acclimatization.

Main results: 73 ± 9% of nodal segments grew to generate 1.73 ± 1.03 shoots per explant (length: 0.76 ± 0.44 cm). After four weeks in hydroponic system, 46 ± 4 % of micropropagated shoots developed roots, which represents an acceptable and intermediate rate of success, compared to the reported in vitro rooting rates.

Research highlights: Our protocol allowed to obtain micropropagated seedlings in a total timespan of 8 weeks. Our results show that, by utilizing a hydroponic system, traditional protocols to micropropagate Eucalyptus can be substantially enhanced, allowing for improved production dynamics and potentially resulting in better organized seedling manufacturing facilities.

Key words: Woody plants; silviculture; nursery seedlings; rooting methods; hydroponics; acclimatization.

Author contributions: DGAV, LLE and RJJ obtained micropropagated eucalyptus shoots. DGAV, LLE and RJJ rooted shoots in hydroponics system. CJM designed hydroponic systems. TE and RJJ wrote the manuscript. TE, AG and RJJ experimental manuscript design. PASI and AG critical revision of manuscript. PASI English revision of manuscript. TE, AG and RJJ had the initial idea of the manuscript.

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Introduction

Eucalyptus is a genus of woody plants which has gained great relevance in the last decades as a rapid source of cellulose pulp and solid wood (Albaugh *et al*., 2016). *Eucalyptus* species currently lead the hardwood production market globally, including the countries of the Southern region of South America. Specifically, in the case of Argentina, of the 1.1 million hectares forested, 22% corresponds to *Eucalyptus* (MAGyP, 2014), with an upward trend, being *Eucalyptus grandis* Hill ex Maiden the most cultivated *Eucalyptus* species in the area (Frangi *et al.,* 2016).

The traditional method for obtaining seedlings is the germination of seeds originated in a seed orchard (Skolmen & Ledig, 1990). However, nurseries have obtained -through crossbreeding- elite specimens whose special traits should be kept (de Assiss, 2001). With this objective, the forestry industry is in a process of transition towards the cultivation of clones. Thus, rapid vegetative propagation techniques for producing *E. grandis* clones are required by forestry companies nationally and worldwide in a strategic path towards enhancement of overall productivity (FAO, 2008).

As conventional macropropagation schemes limit the productivity of eucalyptus plantations due to inefficient rooting rates or excessive rooting timespans (Trueman *et al.*, 2018), *in vitro* propagation techniques are replacing the macropropagation protocols (Nakhooda & Jain, 2016). Shoot culture is probably the most common technique for *E. grandis* micropropagation (Nakhooda & Jain, 2016; Trueman *et al.,* 2018,) and comprises the following stages, each of which takes between 2 and 6 weeks: shoot culture, shoot multiplication, shoot elongation, root formation and acclimatization (Trueman *et al.,* 2018), with an average time required of around 6 months. A possible way to shorten the duration of these protocols is the condensation of some stages in the same time period. For example, rooting of micropropagated shoots could be combined with the acclimatization in a hydroponic system.

The culture of *Eucalyptus* in hydroponic systems has been consistently reported for decades with different purposes: phytoremediation (Iori *et al.,* 2017), drought or salinity stress studies (Nawaz *et al.,* 2016), fertilization experiments (Niu *et al.,* 2015) or in combination with different microorganisms (Egerton-Warburton, 2015). Interestingly, hydroponic systems have never been employed for rooting of micropropagated *Eucalyptus* shoots and, to our knowledge, it has not been reported in other tree species either. It has been described, however, for rooting micropropagated shoots in shrub species such as *Gypsophila paniculata* (Wang *et al.,* 2013), *Rubus fruticosus* (Clapa *et al.,* 2013), *Rosa hybrida* (Clapa *et al.,* 2013), or *Rhododendron sp*. (Zaytseva *et al.,* 2018) and herbaceous species such as *Spathiphyllum sp.* (Dewir *et al.,* 2005) or *Solanum tuberosum* (Piao *et al.,* 2004).

The aim of the present work was to develop a new and faster micropropagation protocol for *Eucalyptus grandis* that simultaneously would allow the direct rooting and acclimatization of the micropropagated shoots in a hydroponic system.

Materials and methods

Eucalyptus grandis growth in a hydroponic system

Fifty mg of the seed mix of *Eucalyptus grandis*, provided by Paul Forestal (CO Nº4081) from clonal seed orchard CIEF-Paul Forestal, were incubated in Petri dishes with a filter paper moistened with 10 ml of a nutritive solution (Javot *et al.,* 2003), at 23 ± 2 °C under dark conditions during 7 days. Afterwards, the dishes were incubated under 16:8 h (L:D) photoperiod with a light intensity level of 150 ± 10 µmol photon $m^{-2}s^{-1}$ f and 60% humidity for three days. Then, 21 seedlings were transplanted into aerated hydroponic culture containers, which had styrofoam lids (36 x 15 x 2 cm) with circular holes, that were floating on a basin filled with 5L of the nutritive solution (seedlings were secured in position using 3 mm-thick polyurethane discs). Systems were incubated in a growth chamber for three months under the same conditions described above. After three months, seeding size was enough to extract the nodal segment to be cultured *in vitro*.

Shoot initiation of nodal segments

We cut branches from *Eucalyptus grandis* seedlings grown in the hydroponic system as described above. First, we eliminated the leaves and the apical section of the branches, which were later carefully washed with a soap solution and distilled water, and were cut again into smaller branches with two or three nodal segments (Fig. 1A). The branches were disinfected in a solution with 24 g l^{-1} of sodium hypochlorite for 5 min under aseptic conditions, followed by three 5 min rinses with distilled sterile water. Finally, the disinfected branches were cut into 3 cm long nodal segments and at least two axillary buds, removing a basal and apical section of 0.5 cm that could be possibly damaged during sterilization. The obtained nodal segments were cultured individually in test tubes that contained 20 ml of initiation medium. The shoot initiation medium consisted

in MS (Murashige & Skoog, 1962) culture medium supplemented with 1 mg l^{-1} 6-benzylaminopurine (BAP), 30 g l^{-1} sucrose solidified with 8 g l^{-1} of bacteriological agar (pH 5.74). As an antioxidant we used 1 g l^{-1} of activated charcoal. The nodal segments were placed in an incubator model I-291PF (Ingelab) and incubated at 25 ± 2 °C under 16:8 h (L:D) photoperiod with a light intensity level of 40 µmol photon $m^{-2}s^{-1}$ for four weeks. Thirty nodal segments were employed in each repetition and three independent experiments were performed. After these four weeks, we calculated the percentage of shoot initiation and measured the number and length of the micropropagated shoots.

Ex vitro rooting and acclimatization in hydroponic system

The micropropagated shoots were cut from the nodal segments and transferred into aerated hydroponic culture containers. The hydroponic system was similar to the already described one, but now the 20 mm-thick styrofoam lids were replaced by a high-impact polystyrene plaque (1 mm thick) while the micropropagated shoots were secured with 3 mm-thick polyurethane discs (Fig. S1 [suppl.]). To induce the *ex vitro* rooting, the micropropagated shoots were dipped for 15 seconds in a solution of 5000 ppm indol butyric acid (IBA) before being transferred to this hydroponic system and maintained for four weeks under the same conditions described before. The hydroponic system was wrapped with plastic film to maintain high humidity and avoid drying of micropropagated shoots. From the second week, holes were made in the plastic film to slowly reduce humidity until reaching the relative humidity of the culture chamber (60 %). Finally, at the end of the fourth week, the plastic wrap was removed and rooted shoots were counted. Three independent hydroponic culture containers were established with 24 micropropagated shoots in each case. The rooted eucalyptus continued its growth in a hydroponic system.

Statistical analysis

All data obtained in this manuscript were analyzed using SPSS software package (version 22.0; SPPS INC., Chicago, IL, USA). We compared the results between independent experiments to check that there were no significant differences between them. Initiation success rate of nodal segments and rooting rate, binomial variables, were analyzed by Generalized Linear Models using Logit as the link function and Binomial as the probability distribution. The length and number of micropropagated shoots from each nodal segment, linear variables, were analyzed by one-way ANOVA.

Results and discussion

To develop our rapid micropropagation protocol, we used nodal segments of seed-derived *E. grandis* seedlings as primary explants (Trueman *et al.,* 2018). Micropropagation using nodal segments is based on the existence of axillary buds at the base of each leaf axil and their ability to grow. We supplemented MS culture medium with 1 mg BA to promote the growth of axillary buds, obtaining a success rate of 73 ± 9% (Fig. 1B), shoots that grew. Concentrations between 0.1 and 1.5 mg l^{-1} of BAP are normally used in the nodal segment culture of *Eucalyptus* (Trueman *et al.,* 2018). Our initial success rate is strong compared to previous research: Watt *et al.,* 2003 obtained success rates between 67% and 78% at the initiation of nodal segments of three different genotypes of *E. grandis;* in the case of hybrids of *E. grandis* with other *Eucalyptus* species, the success rate varied between 18% (*E. grandis* x *E. camaldulensis*) and 91.5% (*E. grandis x E. urophylla*) (Watt *et al.,* 2003; Watt, 2014). Moreover, as *Eucalyptus* has multiple buds within each leaf axil (Jasrai *et al*., 1999), we obtained an average of 1.73 ± 1.03 micropropagated shoots in each nodal segment, with a length of 0.76 ± 0.44 cm (Fig. 1C). Other authors have obtained between 0,5 and 3 shoots per nodal segment in hybrids of *Eucalyptus grandis* (Watt *et al.,* 1995; Borges *et al.* 2011). The next step among *Eucalyptus* micropropagation protocols, involves the multiplication and/or elongation of the obtained shoots (2-6 weeks and 3-4 week of duration respectively), followed by their rooting (Table S1 [suppl.]; Trueman *et al.*, 2018). In our protocol, however, suppressing these two steps and directly moving forward to the rooting of micropropagated shoots (in a hydroponic system) reduced the extension of the whole protocol to 8 weeks. It should be noted, nonetheless, that a reduction in the number of total plants obtained was detected. Remarkably, there are no previous reports in the literature where *Eucalyptus* micropropagated shoots had been successfully rooted in a hydroponic system.

The rooting rate that we obtained was 46 ± 4 % after 4 weeks of incubation (Figs. 1D and 1E). Not surprisingly, the contact between the micropropagated shoots and the hydroponic solution was key to achieve a good shoot rooting. The short length of the micropropagated shoots (0.76 ± 0.44 cm, Fig. 1C) made this contact difficult, so the replacement of the styrofoam lid (20 mm) by a 1 mm thick high-impact polystyrene plaque was critical to the success of the rooting process.

With the aim of comparing the rooting rate obtained here with the rates published elsewhere, it is important to note that the latter ones tend to closely relate with the *E. grandis* or *E. grandis* hybrid genotypes utilized. From the gathered literature, the *in vitro* rooting rate varies between 100%, in genotypes of *E. grandis* (Na-

Figure 1. A) Initial nodal segment of *Eucalyptus grandis* used to obtain micropropagated shoots. B) Nodal segment after 4 weeks of *in vitro* culture. C) Micropropagated shoots obtained after 4 weeks *in vitro* culture. D) Hydroponic system used for *ex vitro* rooting and acclimatization of micropropagated shoots. E) Rooted plantlet after 4 weeks of the acclimatization. F) Rooted plantlet after 8 weeks of the acclimatization. G) Rooted plantlet after 12 weeks of the acclimatization.

khooda *et al.,* 2011; 2014), to 35% in a hybrid of *E. grandis x E. urophylla* (de Oliveira *et al.,* 2017). Other authors have obtained a range of varying *in vitro* rooting rates: 90% or 80% in genotypes of *E. grandis* (Hajari *et al.,* 2006; Almeida *et al.,* 2015), 36.7% to 90% in hybrids of *E. grandis x E. nitens* (Mokotedi *et al.,* 2000; Watt, 2014), 35% to 90.0% in hybrids of *E. grandis x E. urophylla* (Jones & van Staden, 1994; Yang *et al.,* 1995; Whitehouse *et al.,* 2002; Hajari *et al.,* 2006; Watt, 2014;) and 70% in a hybrid of *E. grandis x E. camaldulensis* (Whitehouse *et al.,* 2002). Hence, the rooting rate that we have obtained (46±4%) was lower-intermediate compared to the rates already published.

The main advantage of rooting in a hydroponic system is that the rooting and the acclimatization can be achieved simultaneously, while the *Eucalyptus* shoots rooted *in vitro* (conventional protocols) must be acclimatized in a further additional step. By performing these two steps in a combined manner, we reduced the overall duration of the protocol by 4 weeks. Indeed, the acclimatization success rates are also typically related to the micropropagated genotype utilized, varying between 20% to 100% (Trueman *et al.,* 2018). Thus, the acclimatization not only prolongs the time of the micropropagation protocol, but also reduces the total number of seedlings obtained. In the case of our method, however, all the shoots rooted in the hydroponic system survived and continued their normal growth (Figs. 1F and 1G).

In conclusion, the use of a hydroponic system for *ex vitro* rooting of *E. grandis* micropropagated shoots allowed us to obtain seedlings faster than conventional protocols and with an acceptable success rate. This rapid micropropagation protocol has the potential of being useful for propagating elite genotypes with commercial purposes or for performing *Eucalyptus* physiological studies.

References

Albaugh TJ, Albaugh JM, Fox TR, Allen HL, Rubilar RA, Trichet P, Loustau D, Linder S, 2016. Tamm Review: light use efficiency and carbon storage in nutrient and water experiments on major forest plantation species. For Ecol Manag 376: 333-342. https://doi.org/10.1016/j.foreco.2016.05.031

Almeida MR, Bastiani D, Gaeta ML, Mariath JEA, Costa F, Retallick J, Nolan L, Tai HH, Strömvik MV, Fett-Neto AG 2015. Comparative transcriptional analysis provides new insights into the molecular basis of adventitious rooting recalcitrance in Eucalyptus. Plant Sci 239: 155-165. https://doi.org/10.1016/j.plantsci.2015.07.022

Booth TH, 2013. Eucalypt plantations and climate change. For Ecol Manag 301: 28-34. https://doi.org/10.1016/j.foreco.2012.04.004

Borges SR, Xavier A, Oliveira LS, Lopes AP, Otoni WC 2011. Multiplicação in vitro de clones híbridos de Eucalyptus globulus. Rev Árvore 35: 173-182. https://doi.org/10.1590/S0100-67622011000200001

Clapa D, Fira A, Joshee N, 2013. An efficient ex vitro rooting and acclimatization method for horticultural plants using float hydroculture. Hort Sci 48: 1159-1167. https://doi.org/10.21273/HORTSCI.48.9.1159

de Assiss TF, 2001. Evolution of technology for cloning Eucalyptus in large scale. Developing the Eucalypt of the Future. IUFRO, Chile. 1-6.

Dewir YH, Chakrabarty D, Ali MB, Hahn EJ, Paek KY, 2005. Effects of hydroponic solution EC, substrates, PPF and nutrient scheduling on growth and photosynthetic competence during acclimatization of micropropagated Spathiphyllum plantlets. Plant Growth Reg 46: 241-251. https://doi.org/10.1007/s10725-005-0161-1

Egerton-Warburton L, 2015. Aluminum-tolerant pisolithus ectomycorrhizas confer increased growth, mineral nutrition, and metal tolerance to eucalyptus in acidic mine spoil. Appl Environ Soil Sci 2015:803821. https://doi.org/10.1155/2015/803821

FAO, 2008. Eucalyptus Universalis. Cultivated Eucalypt Forests Global Map. FAO Department of Forestry, Rome, V. 1.0.

Frangi JL, Perez CA, Goya JF, Arturi M, 2016. Experimental model of Eucalyptus grandis plantation in Concordia, Entre Rios. Bosque 37: 191-204. https://doi.org/10.4067/S0717-92002016000100018

Hajari E, Watt MP, Mycock DJ, McAlister B, 2006. Plant recognition from induced callus of improved Eucalyptus clones. S Afr J Bot 72: 195-201. https://doi.org/10.1016/j.sajb.2005.07.003

Iori V, Pietrini F, Bianconi D, Mughini G, Massacci A, Zacchini M (2017). Analysis of biometric, physiological, and biochemical traits to evaluate the cadmium phytoremediation ability of eucalypt plants under hydroponics. IForest 10: 416-421. https://doi.org/10.3832/ifor2129-009

Jasrai YT, Remakanthan A, Pandya CH, Subramani J, Bhatt DP, 1999. Multiplication of Eucalyptus citriodora (L.) Hook through shoot bud induction on the internodal portions of mature tree explants. J Plant Biochem Biotechnol 8: 103-104. https://doi.org/10.1007/BF03263067

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner, AR, Bouchez D, Maurel C, 2003. Role of a single aquaporin isoform in root water uptake. The Plant Cell 15: 509-522. https://doi.org/10.1105/tpc.008888

Jones NB, van Staden J, 1994. Micropropagation and establishment of Eucalyptus grandis hybrids. S Afr J Bot 60: 122-126. https://doi.org/10.1016/S0254-6299(16)30643-3

MAGyP, 2014. Argentina: plantaciones forestales y gestión sostenible. URL http://forestoindustria.magyp.gob.ar/archivos/gestion-forestal-sostenible/publi_ambiental.pdf (Accessed 8 November 2018).

Mokotedi MEO, Watt MP, Pammenter NW, 2000. In vitro rooting and subsequent survival of two clones of a cold-tolerant Eucalyptus grandis × E. nitens hybrid. Hort Sci 35: 1163-1165. https://doi.org/10.21273/HORTSCI.35.6.1163

Murashige T, Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nakhooda M, Jain SM, 2016. A review of Eucalyptus propagation and conservation. Propag Ornam Plants 16: 101-119.

Nakhooda M, Watt MP, Mycock D, 2011. Auxin stability and accumulation during in vitro shoot morphogenesis influences subsequent root induction and development in Eucalyptus grandis. Plant Growth Regul 65: 263-271. https://doi.org/10.1007/s10725-011-9597-7

Nakhooda M, Rambaran N, Mokotedi MEO, Watt MP, 2014. Sensitivity and response of Eucalyptus grandis × E. nitens cuttings to auxin treatment for rooting is consistent with, and can be predicted by in vitro morphogenesis. J Hortic Sci Biotechnol 89: 215-220. https://doi.org/10.1080/14620316.2014.11513071

Nawaz MF, Gul S, Tanvir MA, Akhtar J, Chaudary S, Ahmad I, 2016. Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (Eucalyptus camaldulensis Dehnh.). Turk J Agric For 40: 425-432. https://doi.org/10.3906/tar-1407-73

Niu F, Zhang D, Li Z, Van Iersel MW, Alem P, 2015. Morphological response of eucalypts seedlings to phosphorus supply through hydroponic system. Sci Hort 194: 295-303. https://doi.org/10.1016/j.scienta.2015.08.029

de Oliveira C, Degenhardt-Goldbach J, Bettencourt GMF, Amano E, Franciscon L, Quoirin M 2017. Micropropagation of Eucalyptus grandis x E. urophylla AEC 224 clone. J For Res 28: 29-39. https://doi.org/10.1007/s11676-016-0282-6

Piao XC, Chakrabarty D, Hahn EJ, Paek KY, 2004. The growth and photosynthetic characteristics of potato (Solanum tuberosum L.) plantlets as affected by hydroponic solution pH and EC, light, and CO2. J Am Soc Hort Sci 129: 100-105. https://doi.org/10.21273/JASHS.129.1.0100

Skolmen RG, Ledig FT, 1990. Eucalyptus globulus (Labill) Bluegum eucalyptus. In: Burns RM; Honkala BH, (Eds.) Silvics of North America 2. Forest service, Washington. 299-304.

Trueman SJ, Hung CD, Wendling I, 2018. Tissue culture of Corymbia and Eucalyptus. Forests 9: 84. https://doi.org/10.3390/f9020084

Wang SM, Piao XC, Park SY, Lian ML, 2013. Improved micropropagation of Gypsophila paniculata with bioreactor and factors affecting ex vitro rooting in microponic system. In vitro Cell Dev Biol-Plant 49: 70-78. https://doi.org/10.1007/s11627-012-9464-x

Watt MP, 2014. Genotypic-unspecific protocols for the commercial micropropagation of Eucalyptus grandis × nitens and E. grandis × urophylla. Turk J Agric For 38: 125-133. https://doi.org/10.3906/tar-1304-83

Watt MP, Duncan EA, Ing M, Blakeway FC, Herman B, 1995. Field performance of micropropagated and macropropagated Eucalyptus hybrids. South Afr For J 173: 17-21. https://doi.org/10.1080/00382167.1995.9629685

Watt MP, Berjak P, Makhathini A, Blakeway F (2003). In vitro field collection techniques for Eucalyptus micropropagation. Plant Cell Tissue Organ Cult 75: 233-240. https://doi.org/10.1023/A:1025819826742

Whitehouse AB, Marks TR, Edwards GA, 2002. Control of hyperhydricity in Eucalyptus axillary shoot cultures grown in liquid medium. Plant Cell Tissue Organ Cult 71: 245-252. https://doi.org/10.1023/A:1020360120020

Yang JC, Chung JD, Chen ZZ, 1995. Vegetative propagation of adult Eucalyptus grandis × urophylla and comparison of growth between micropropagated plantlets and rooted cuttings. Plant Cell Rep 15: 170-173. https://doi.org/10.1007/BF00193713

Zaytseva YG, Ambros EV, Novikova TI, 2018. Rooting and acclimatization to ex vitro conditions of regenerants of frost-resistant members of Rhododendron. Turczaninowia 21: 144-152. https://doi.org/10.14258/turczaninowia.21.1.13

Zobel BJ, 1993. Clonal forestry in the eucalypts. In: Ahuja MR; Libby WJ (Eds.) Clonal Forestry II. Springer, Berlin. 139-148. https://doi.org/10.1007/978-3-642-84813-1_7