Plant Cell Culture & Micropropagation

http://pccm.ufla.br/index.php/plantcellculturemicropropagation/index

In vitro multiplication of *Cedrela fissilis* Vell.: A threatened brazilian hardwood forest tree

Yasmim Smarsaro Bonfá[1], Vitor de Laia Nascimento[2], Elias Terra Werner[1*]

ABSTRACT

Cedrela fissilis Vell. is a valuable wood species from Atlantic Forest and is at risk of extinction. Therefore, a protocol for clonal multiplication was developed for *C. fissilis* using different explants from *in vitro* seedlings. The seeds were germinated in total or half-strength MS and B5 basal media. The half-strength MS medium was chosen based on its high germination rate (82 %) and germination speed index (1.218). Shoot tips, nodal segments and cotyledonary nodes explants were extracted from the *in vitro* seedlings and cultivated for shoot induction in MS medium supplemented with KIN or BAP (0, 0.5, 2 and 4 µM). The highest number (2.2) and length (3.84 cm) of shoots were obtained from cotyledonary nodes cultured in the absence of cytokinin. For rooting, the MS medium was supplemented with IBA or NAA (0, 1 and 3 µM). Rooting was induced in all auxin treatments. The highest number of roots per explant was observed in the treatment supplemented with 3µM IBA (2.54). The present study provides *in vitro* shoot multiplication and root regeneration systems for *C. fissilis*, with great potential to be used for micropropagation, germplasm preservation and genetic transformation.

Index terms: Cedro-rosa; vulnerable; cotyledonary nodes; plant growth regulator; micropropagation.

INTRODUCTION

Cedrella fissilis Vellozo (Meliaceae) popularly known as "Cedro", "Cedro-rosa" or "Acaiacá" (Lorenzi, 2008) is a forest species widely dispersed throughout Brazil (Stefano; Calazans; Sakuragui, 2015). It has a light wood color and has high economic value with wide commercial use (Carvalho, 1994; Rizzini; Mors, 1976). This species is the target of extractivism and exploitation leading to deforestation (Ruiz-Filho et al., 2004). *C. fissilis* is currently at risk of extinction, categorized as vulnerable (Centro Nacional de Conservação da Flora - CNCFlora, 2013; Flores; Souza; Coelho, 2017; Barstow, 2018).

Due to deforestation and, consequently, the ecological imbalance, Barbosa and Barbosa (2007) postulate that forest restoration is needed and it should be as close as possible to the original forests, to reestablish their structures and functions. The forest restoration in Brazil is also need for reasons such as: compliance with the Forest Code (Brasil, 2012), which created instruments for regularization and environmental restoration; and the Brazil's goal to restore and reforest 12 million hectares by 2030 propose in the Nationally Determined Contribution (NDC) to the Paris Agreement (United Nations Framework Convention on Climate Change - UNFCCC, 2016). A worrying factor for forest restoration in Brazil is the drastic reduction in the last few years in the production of seedlings of native species of the Atlantic Forest (Instituto de Pesquisa Econômica Aplicada - IPEA, 2015; SOS Mata Atlântica foundation, 2014).

Therefore, global needs in the face of excessive demand for products from native plant species place biotechnological techniques as the front line for gains in yield and sustainability (Watanabe; Raman 1997). *In vitro* propagation techniques have been developed to *C. fissilis* plant regeneration through somatic embryogenesis (Vila et al., 2009) or organogenesis (Nunes et al., 2002; 2007; Aragão et al., 2016; 2017; Oliveira et al., 2020), leading to progress in plant biotechnology this tree over the last decades. These studies on the *in vitro* propagation of *C. fissilis* show that besides offering the possibility of homogeneous large-scale plantlet production for

https://doi.org/10.46526/pccm.2021.v17.165
Received in April 6, 2021 and approved in May 26, 2021
[1]Universidade Federal do Espírito Santo/UFES, Campus Goiabeiras, Vitória, ES, Brasil
[2]Universidade Federal de Lavras/UFLA, Departamento de Biologia, Setor de Fisiologia Vegetal, Lavras, MG, Brasil
*Corresponding author: eliaswerner12@gmail.com

commercial plantations, it is an effective option of biotechnological approaches for the conservation and preservation of this plant species.

However, new approaches for *C. fissilis in vitro* propagation are necessary for greater efficiency in shoot multiplication and root induction, and methodologies of acclimatization. *In vitro* propagation is affected by several factors, such as mineral nutrition in culture medium, source of explants, type and concentration of plant growth regulators (PGRs) and *in vitro* environmental conditions (Oliveira et al., 2020).

The most common culture medium used for *in vitro* seed germination and *C. fissilis* micropropagation is the Murashige and Skoog medium (MS) (Murashige; Skoog, 1962) (Nunes et al., 2002; 2007; Vila et al., 2009; Aragão et al., 2016; 2017; Oliveira et al., 2020), only Nunes et al. (2002) tested different culture medium [MS, SH (Schenk; Hildebrandt, 1972), KM (Kao; Michayluk, 1975), AR (Anderson, 1978), W (White, 1963), WPM (Lloyd; McCown, 1981) and B5] for shoot multiplication. Therefore, testing other culture medium with diluted nutritional concentrations, such as the Gamborg B5 (B5) medium (Gamborg; Miller and Ojima, 1968), or different medium strength, may be a viable option for seed germination.

In vitro seedlings of *C. fissilis* may be used as explant source. Most all studies performed with *C. fissilis* used shoot tips and cotyledonary nodes segments for *in vitro* shoot multiplication (Nunes et al., 2002; 2007; Aragão et al., 2016; 2017; Oliveira et al., 2020). The present study also used the nodal segment as an alternative because it presents axillary buds with great possibility of multiplication.

Among PGRs, 6-benzylaminopurine (BA) is the main cytokinins applied to *in vitro* shoot multiplication in *C. fissilis* (Nunes et al., 2002; Aragão et al., 2016; Oliveira et al., 2020). For rooting, Nunes et al. (2002; 2007) reported the formation of 5-6 roots per explant using naphthaleneacetic acid (NAA), after 35 days. These results showed the need for more tests with PGRs for a high multiplication and production of *C. fissilis in vitro* plantlets.

Therefore, the objective of this study is to propose a protocol for *in vitro* propagation of *C. fissilis* using the micropropagation technique. The steps for its implementation were conducted seeking to increase the yield, healthy plantlets generation, and the cost-benefit, considering the best culture medium for *in vitro* establishment, as well as the explant, PGRs-types and, concentrations for multiplication and rooting.

MATERIAL AND METHODS

In vitro seed germination

Seeds of *Cedrela fissilis* Vell. (batch CFI001SN) were donated by "Flora Tietê" - Forest Recovery Association - Penápolis - SP (21° 25′ 15″ S, 50° 4′ 41″ W). The winged seeds were mechanically removed and then washed in running water with pH-neutral detergent for ten minutes. Under aseptic conditions in the laminar flow chamber, the seeds were submerged in 70% alcohol with three drops of Tween 20® for one min, followed by sodium hypochlorite (2.5% active chlorine) with five drops of Tween 20® for 75 min under constant agitation, and then rinsed five times in sterile distilled water (adapted from Nunes et al., 2002).

The seeds were subsequently placed in test tubes (25 x 150 mm) (one seed/tube) containing 10 mL of full or half-strength Murashige and Skoog basal medium (MS, Sigma®) or full- and half-strength Gamborg B5 basal medium (B5, Sigma®). The media were supplemented with 30 gL^{-1} (full) and 15 gL^{-1} (half) sucrose (Sigma®), and 7.5 gL^{-1} agar (Sigma®) as a gelling agent, pH adjusted with 0.1 $molL^{-1}$ NaOH and autoclaved at 121 °C for 21 min. All explants were cultured at 25±2°C under cool-white fluorescent light by 16 h photoperiod with light intensity of 25.2 $\mu molL\ m^{-2}\ s^{-1}$.

After four weeks of inoculation the germination rate (%G) (Maguire, 1962), germination speed index (GSI) (Silva et al., 2019), fresh and dry mass, number of leaves, shoot and root length were evaluated. The experiment was conducted in a completely randomized design with four treatments and five replicates with ten seeds/replicate.

In vitro shoot multiplication and root induction

Different explants were extracted from the aseptically grown *in vitro* plantlets with four weeks for *in vitro* multiplication. The experimental design was a completely randomized factorial design of 3x2x4 (explants x cytokinins x concentrations), three types of explants (shoot tips, nodal segments, cotyledonary nodes), two types of cytokinins (kinetin-KIN and 6-benzylaminopurine-BAP) (Sigma®), and four cytokinins concentrations (0.0, 0.5, 2.0, and 4.0 µM), with three replicates with

five explants/replicate. The explants were placed in tubes (one explant/tube) containing 10 mL of MS medium. After four weeks of cultivation, the number of shoots per explant, shoot length and rooting rate were evaluated.

Shoots obtained with number greater than 2.0 shoots/explant and length greater than 2.0 cm/explant were used for root induction. The experiment was conducted in a completely randomized design with two auxins tested, indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) (Sigma®), and three concentrations (0.0, 1.0, and 3.0 µM), with three replicates with ten explant/replicate. The explants were placed in tubes (one explant/tube) containing 10 mL of MS medium. After four weeks of cultivation the number of root per explant, rooting rate and survival were measured. The conditions of the growth room remained the same as described above.

Acclimatization

The *in vitro* rooted plantlets with the number of roots greater than 2.0 roots/plantlets and length greater than 2.0 cm/plantlets were removed from test tubes and the roots washed with running water to remove excess medium. Subsequently, they were transferred to plastic pots (50 ml) containing a sterilized commercial substrate (Carolina Soil®), covered by transparent polyvinylchloride (PVC) film and watered weekly. These were incubated in the same condition of the growth room as described above and after four weeks the PVC film was removed. After four and eight weeks the survival rate was evaluated.

Statistical analysis

The results for each experiment were subjected to Shapiro-Wilk tests for residual normality and Bartlett for homogeneity between variances. The collected data were statistically evaluated based on analysis of variance (ANOVA) and the means were then compared by the Tukey test with a significance level of 5%. Data analysis was performed using SISVAR statistical software (Ferreira, 2014).

RESULTS AND DISCUSSION

In vitro multiplication of *C. fissilis* was developed within three months. Each stage of the multiplication process is shown in Figure 1. The results are described below.

***In vitro* seed germination**

During *in vitro* seed germination of *C. fissilis,* no differences have been observed for most of the germination parameters analyzed, with the exception of GSI and dry mass (Table 1). Although, a highest uniform visual pattern of the seedlings was observed in full-strength MS medium (MS) when compared to the others media-treatments (Figure 2).

In this study, seed germination in half-strength MS medium (MS½), and in absence of PGR was initiated 3-4 days after seed inoculation. In this condition, the germination percentage was 82%, GSI 1,21, total fresh mass 0.305 g, total dry mass of 0.035 g, number of leaves 2.7, shoot length 4.83 cm and root length 5.74 cm. These results were lower than those reported by Nunes et al. (2002, 2007) using the full-strength MS culture medium. However, in the present study, no significant difference was found in the germination percentage among the culture media tested (Table 1). The use of MS½ medium for germination and *in vitro* establishment of *C. fissilis*, allows a cost reduction as well as a decrease in the time required for the seedlings to reach the extraction point of the explants.

Despite the MS formulation used, being original (full-strength) or fractionated (half-strength), both tested media can be used for *in vitro* seed germination of *C. fissilis* without difference in the rate of germinated plants. However, the GSI had better results in the medium with reduced salts concentrations. These results are due to the rapid imbibition of the seeds in medium with low salts concentrations, considering the difference between the water potentials of the external and internal environments to the seed membranes, which provide the water movement, from the highest to the lowest water potential (Castro; Bradford; Hilhorst, 2004). This fact might be justified by the lower salt concentration of half-strengths MS and B5 media, especially nitrogen, when compared to the MS culture medium tested. Bhojwani and Razdan (1996) and Pereira et al. (2000) reported that for some native species, medium with reduced salts concentration favor better plant growth *in vitro* production, and for some species the total salts concentration in commercial formulations is toxic or unnecessarily high.

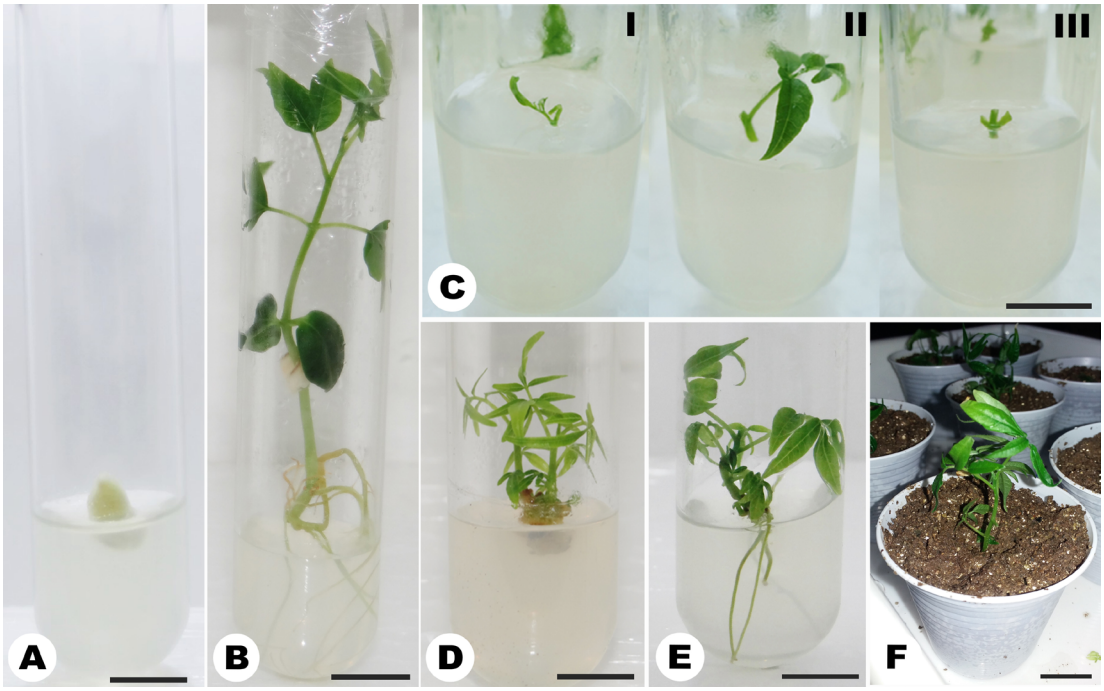

Figure 1: *In vitro* germination and multiplication of *Cedrela fissilis*. (A) *in vitro* germination; (B) 30-day plantlet, explant extraction point; (C) explants extracted (I: shoot tips; II: nodal; III: cotyledonary); (D) *in vitro* multiplication; (E) *in vitro* rooting; (F) acclimatization. Scale = 1 cm.

Table 1: Values for germination percentage (% G), germination speed index (GSI), total fresh mass (FM), total dry mass (DM), number of leaves (NL), shoot length (SL) and root length (RL) of *Cedrela fissilis* after 30 days of inoculation in different culture media.

Culture medium	%G	GSI	FM (g)	DM (g)	NL	SL (cm)	**RL (cm)**
MS	72a	1.096b	0.300a	0.039a	2.708a	5.153a	5.138a
MS 1/2	82a	1.218a	0.305a	0.035ab	2.706a	4.830a	5.744a
B5	76a	1.140ab	0.288a	0.037ab	3.081a	5.532a	6.538a
B5 1/2	78a	1.210a	0.242a	0.033b	3.038a	5.446a	4.572a
CV (%)	11.06	5.36	45.91	25.96	38.05	36.34	61.55

Averages followed by the same letter in the column do not differ by Tukey's test at 5% probability.

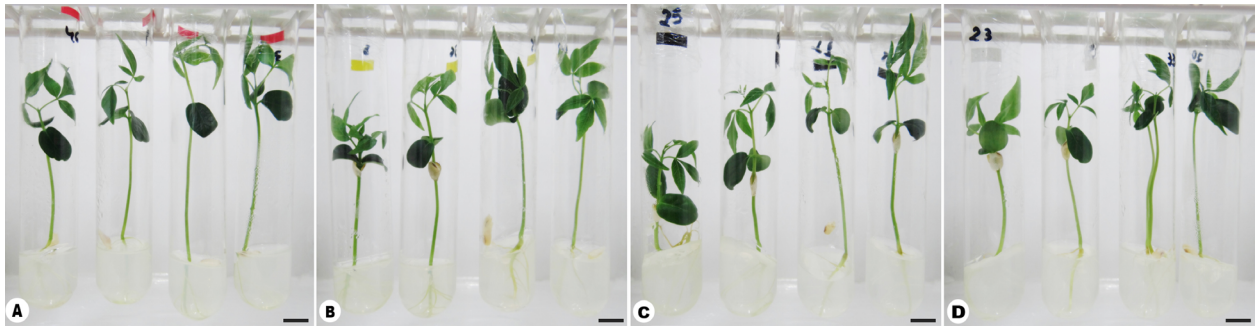

Figure 2: Visual pattern of the *Cedrela fissilis* seedlings in the different cultivation media: (A) MS, (B) half-strength MS, (C) B5, (D) half-strength B5. Scale = 1 cm.

The cost of each culture medium must be considered, even though this technology has several advantages, *in vitro* seedling production still has a high cost (Ribeiro et al., 2013). Considering the above, half-strength MS medium has a lower cost when compared to the other mediums tested. In addition, even if it does not present differences in number of leaves or shoot and root length, MS½ provides adequate plant material for the next stages of *in vitro* propagation (micropropagation).

In vitro shoot multiplication and root induction

There was no significant triple interaction among the factors evaluated. There was significant effect for double interaction between explants and cytokinin-concentrations (Figure 3).

Multiple shoots were induced from different types of the explants cultured in MS medium supplemented in the presence and absence (control) of cytokinins. The shoot multiplication was observed within a week of culture initiation and prominent shoots were visible after two weeks. The highest number of shoots (2.2) was observed in cotyledonary nodes cultured in MS medium regardless of cytokinin (Figure 3A). In the shoot tips explant, the highest number of shoots (1.6) was obtained in the absence of cytokinins (control). In nodal segments an increase in the number of shoots was observed only in the treatment with lower cytokinin concentration (0.5 µM), with 1.9 shoots per explant. These results indicate that the presence of cytokinins did not favor an increase in the number of shoots, and it is questioned if the use is essential for *C. fissilis*.

Shoots obtained from cotyledonary nodes as explant were longer (3.84 cm) than from the other explants tested, which did not present lenght greater than 2 cm (Figure 3B). In addition to the best results found for cotyledonary nodes, it was also possible to observe the morphological homogeneity of shoots obtained from this segment compared to other explants tested (Figure 4).

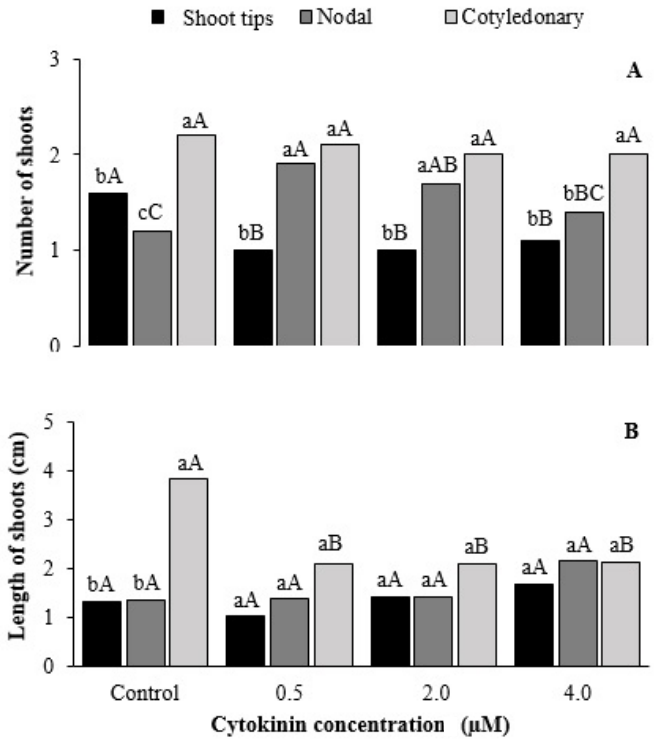

Figure 3: Number (A) and length (B) of shoots obtained from shoot tips, nodal segments and cotyledonary nodes explants of *Cedrela fissilis* under the influence of different cytokinin concentrations after 30 days of *in vitro* cultivation. Capital letters indicate significant differences between different concentrations, lower case letters indicate significant differences between different types of explants in the same treatment according to the Tukey test at 5% probability.

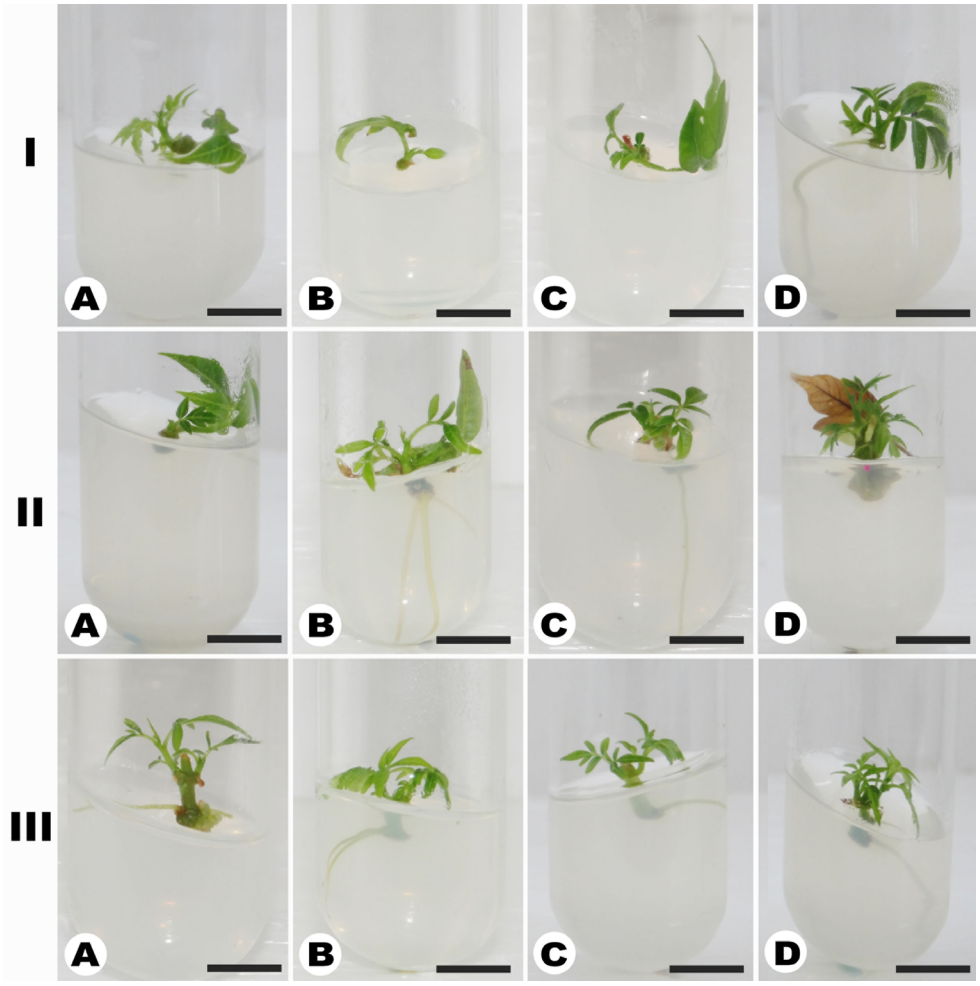

Figure 4: Morphological pattern of shoots obtained form *C. fissilis* shoot tips (I) nodal segments (II) and cotyledonary nodes (III) at different cytokinin concentration [(A) 0 µM; (B) 0.5 µM; (C) 2 µM and (D) 4 µM], after 30 days of *in vitro* cultivation. Scale = 1 cm.

Similar studies using the same explant sources evaluated here were performed by Nunes et al. (2002; 2007), and indicate that the association of auxins and cytokinins may inhibited the shoot multiplication. The highest number of nodes per explant (5.5) and higher shoot elongation (>3.0 cm), which could then be excised for further multiplication and production of new plantlets, was achieved at 2.5 µM BAP from cotyledonary nodes (Nunes et al., 2002; 2007). Thus, this balance can act intensely on the apical dominance exerted by their endogenous ratio and allow a greater number and length of shoots.

Aragão et al. (2016) also described that for *in vitro* shoot multiplication of *C. fissilis*, the shoots obtained from cotyledonary segments cultured in the presence or absence of BA were more vigorous and longer compared to those obtained from nodal segments. In contrast, these authors also reported that nodal segments cultured in the presence 2.5 µM BA exhibited significantly increased shoots per explant in comparison to nodal segments cultured without BA.

Genotypic effect on shoot multiplication and elongation has been described in many species and may be partly due to differences in endogenous hormone levels (Pellegrineschi, 1997; Schween; Schwenkel, 2003). The ratio between endogenous cytokinin and auxin may explain the higher values found for cotyledonary

explant in the control treatment. This fact allows us to consider the performance of apical dominance, since the increase of exogenous cytokinin, even at a small concentration, was enough to cause the growth of shoots to half, altering the auxin/cytokinin balance. However, the ratio over cytokinin did not promote a proportional increase in the number of lateral buds.

Morphologically the cotyledonary node and nodal segment are composed of two axillary buds, a fact that allows at least the emergence of two new shoots. The shoot tips are composed of only one bud, allowing one new bud as a minimum number. This fact, justify the higher numbers of the shoots per explant in the cotyledonary node and nodal segment because the higher number of meristems.

Part of the obtained shoots presents the emergence of roots without the addition of PGRs. This observation shows some rhizogenic capacity of *C. fissilis* tissues, mainly in shoots obtained from cotyledonary nodes. The shoots obtained from this explant source presented a higher rooting rate (45%), in comparison to shoot tips (20%) and nodal segments (25%).

All PGRs-treatments tested favored rooting (Figure 5). It was observed that the rooting rate tended to increase with increased concentration of exogenous auxin concentrations and with the alteration of the type of auxin supplied. The highest rooting rate and number of root per explant were in the MS medium containing IBA 3µM, 63.3% and 2.54 roots per explant, respectively, with 100% of survival. Nunes et al. (2002; 2007) describe the highest rooting percentage (85%) was achieved with 5.0 µM IBA, while the maximum number of roots (5–6) per explant was produced on 1.25–2.5 µM NAA.

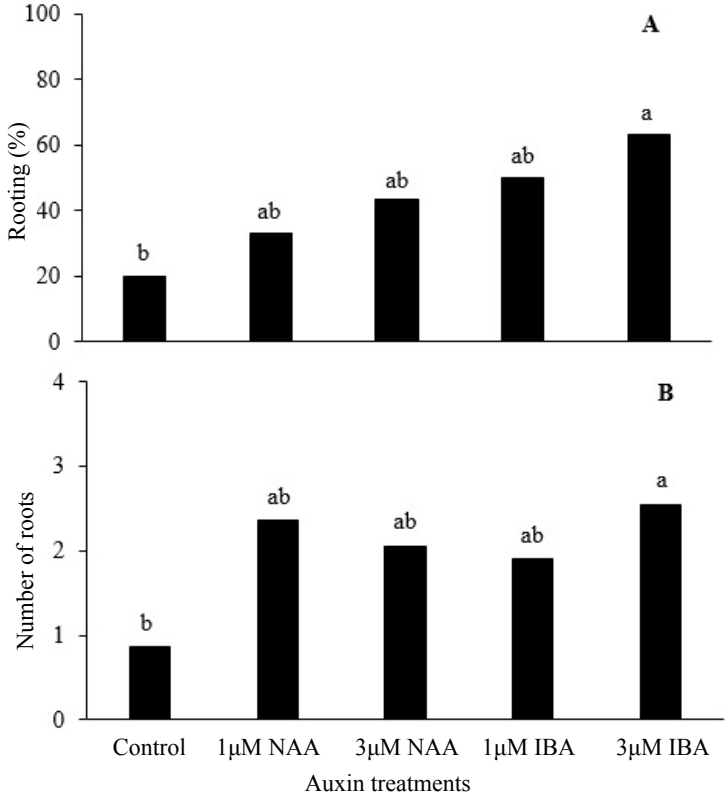

Figure 5: Percentage of rooting (A) and average number of roots per explant (B) of *Cedrela fissilis* shoots submitted to NAA and IBA treatments at different concentrations (0; 1 and 3 µM) after 30 days of *in vitro* cultivation. Columns with the same letters do not differ according to the Tukey 5% test.

Among the auxins commonly used in tissue culture, IBA has been widely used due to low explant phytotoxicity, providing positive results for *in vitro* rooting, as demonstrated for *Caryocar brasiliense* (Santos et al., 2006), *Ocotea porosa* (Pelegrini et al., 2011) and *Parapiptadenia rigida* (Kielse et al., 2009). However, in general, responses are dependent on genetic material and are influenced by culture medium composition, auxin type and concentrations (Oliveira; Dias; Brondani, 2013).

Interestingly, the roots of *C. fissilis* plantlets usually lack root hair and showed little branched, regardless of the auxin treatment, providing a less adequate root system (Figure 6), generating consequences for the treatment and subsequent transplantation and acclimatization to *ex vitro* conditions.

Acclimatization

Sixty rooted seedlings were taken to the acclimatization stage, and 10% survived the process during the initial stage - with PVC film cover. After four weeks the plantlets did not present increase in the number of leaves or shoot length. With the removal of the PVC film, the plantlets survived for only a period of two weeks. Nunes et al. (2002; 2007) reported 100% survival of plants regenerated in the acclimatization with river sand in seed trays covered with a PVC film for 25 days and for another 90 days after the PVC film was gradually removed and transfer to potting medium consisting of three different substrates, at all stages of acclimatization the plants were kept in the culture room atmosphere (average 70% RH).

Acclimatization may be a limiting factor in the micropropagation process (Rocha et al., 2008), exhibiting low plantlet survival rate after transplantation. This deficiency in the final stage of the *in vitro* propagation process is usually due to the characteristics of the roots formed in the agar-solidified media, which are not functional and exhibit scarce formation of root hair, compromising the acclimatization phase (Pierik, 1990; Tibola et al., 2004).

Silva et al. (2011) reported that a critical issue during *ex vitro* acclimatization is the water loss through transpiration or the formed roots *in vitro* that are not always efficient in absorbing water and nutrients when the plantlets were moved to the substrate. Apparently, the parameters used in the present study to acclimatize *C. fissilis* plantlets need to be modified conform Nunes et al. (2002; 2007) reported. However, even plants with normal morphological appearance may not survive due to several factors, including the stress that plants undergo due to the sudden change in relative humidity; for moving into an autotrophic state, where they need photosynthesis to survive; and/or are subject to attack by pathogenic microorganisms (Tibola et al., 2004).

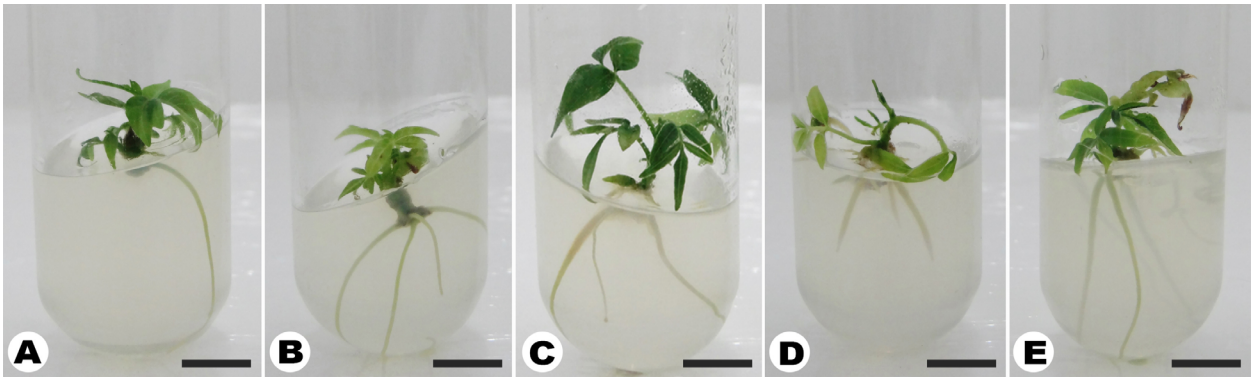

Figure 6: Morphological pattern of the rooted shoots of *Cedrela fissilis* at different types and concentrations of auxin [(A) CONTROL; (B) 1 µM NAA; (C) 3 µM NAA; (D) 1 µM IBA; (E) 3 µM IBA], after 30 days of *in vitro* cultivation. Scale = 1 cm.

CONCLUSIONS

An *in vitro* protocol for seed germination and shoot multiplication of *C. fissilis* in MS medium without any PGR was established. The half-strength culture media were more suitable for germination and initial development of *C. fissilis* seedlings. For *in vitro* shoot multiplication, the cotyledonary node grown in MS medium without cytokinin showed higher number of shoots per explant. For rooting, 3 µM IBA favored the emergence of adventitious roots in most explants, although statistical differences have not been observed among the auxin-treatments. The acclimatization stage did not have results compatible with the micropropagation technique and therefore requires greater attention in cases of commercial production.

ACKNOWLEDGEMENTS

We thank the Federal University of "Espírito Santo" (UFES), CAPES-MEC for the scholarship awarded and "Flora Tietê" - Forest Recovery Association (Penápolis-SP) by donating the seeds of *Cedrela fissilis*.

REFERENCES

ANDERSON, W. C. Tissue culture propagation of Rhododendrons. **In vitro**, 14:334, 1978.

ARAGÃO, V. P. M. et al. Putrescine promotes changes in the endogenous polyamine levels and proteomic profiles to regulate organogenesis in *Cedrela fissilis* Vellozo (Meliaceae). **Plant Cell, Tissue and Organ Culture,** 130:495-505, 2017.

ARAGÃO, V. P. M. et al. *In vitro* organogenesis of *Cedrela fissilis* Vell. (Meliaceae): The involvement of endogenous polyamines and carbohydrates on shoot development. **Plant Cell, Tissue and Organ Culture**, 124:611-620, 2016.

BARBOSA, L. M.; BARBOSA, K. C. Restauração de Matas Ciliares: "A Importância das Interações Fauna e Flora na Restauração de Matas Ciliares". In: BARBOSA, L. M.; SANTOS, J. R. **A Botânica no Brasil:** pesquisa, ensino e políticas públicas ambientais. São Paulo, 2007. p.631-639.

BARSTOW, M. *Cedrela fissilis*. **The IUCN Red List of Threatened Species 2018**: e.T33928A68080477. 2018. Available in: <https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T33928A68080477.en>. Access in: January, 16, 2019.

BHOJWANI, S. S.; RAZDAN, M. K. Clonal Propagation. In: ___. **Plant tissue culture**: theory and practice. Elsevier Science Publishers, 5:483-536, 1996.

BRASIL. Lei nº 12.651, de 25 de maio de 2012. Dispõe sobre a proteção da vegetação nativa. **Diário Oficial da União**: seção 1, Brasília, DF, 2012. Available in: <http://www.planalto.gov.br/ccivil_03/_ato2011-2014/2012/lei/l12651.htm>. Access in: May, 08, 2021.

CARVALHO, P. E. R. **Espécies Florestais Brasileiras**: Recomendações silviculturais, potencialidades e uso da madeira. Paraná: Colombo. EMBRAPA – CNPF/SPI, 1994. 639p.

CASTRO, R. M.; BRADFORD, K. J.; HILHORST, H. W. M. Embebição e reativação do metabolismo. In: FERREIRA, AG; BORGHETTI, F. **Germinação**: do básico ao aplicado. Porto Alegre: Artmed, 2004, p.251-262.

CENTRO NACIONAL DE CONSERVAÇÃO DA FLORA - CNCFlora. **Livro vermelho da flora do Brasil**. MARTINELLI, G.; MORAES, M. A. 1. ed. - Rio de Janeiro: Andrea Jakobsson: Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. 2013. Available in: <http://dspace.jbrj.gov.br/jspui/handle/doc/26>. Access in: January, 14, 2019.

FERREIRA, D. F. Sisvar: A guide for its bootstrap procedures in multiple comparisons. **Ciência e Agrotecnologia**, 38(2):109-112, 2014.

FLORES, T. B.; SOUZA, V. C.; COELHO, R. L. G. Flora do Espírito Santo: Meliaceae. **Rodriguésia**, 68(5):1693-1723, 2017.

GAMBORG, O. L.; MILLER, R. A.; OJIMA, K. Nutrient requirements of suspension cultures of soybean root cells. **Experimental Cell Research**, 50:51-158, 1968.

INSTITUTO DE PESQUISA ECONÔMICA APLICADA - IPEA. **Diagnóstico da Produção de Mudas Florestais Nativas no Brasil: Relatório de Pesquisa**. 2015. Available in: <http://www.lerf.eco.br/img/publicacoes/150507_relatorio_diagnostico_producao.pdf>. Access in: May, 08, 2021.

KAO, K. N.; MICHAYLUK, M. R. Nutritional requirements for growth of *Vicia hajastana* cells and protoplasts at a very low population density in liquid media. **Planta**, 126:105-110, 1975.

KIELSE, P. et al. Regeneração *in vitro* de *Parapiptadenia rigida*. **Ciência Rural**, 39(4):1088-1094, 2009.

LLOYD, G.; MCCOWN, B. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. **International Plant Propagators' Society**, 30:421-427, 1981.

LORENZI, H. **Árvores brasileiras**: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 5.ed. Nova Odessa, 2008, v.1, 384p.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, 2(1):176-177, 1962.

MURASHIGE, T.; SKOOG, F. A. A revised medium for rapid growth and bioassays with tabaco tissue cultures. **Physiologia Plantarum**, 15(3):473-497, 1962.

NUNES, E. C. et al. *In vitro* culture of *Cedrela fissilis* Vellozo (Meliaceae). **Plant Cell, Tissue and Organ Culture**, 70(1):259-268, 2002.

NUNES, E. C. et al. Micropropagation of *Cedrela fissilis* Vell. (Meliaceae). In: JAIN, S. M.; HÄGGMAN, H. (eds.), **Protocols for Micropropagation of Woody Trees and Fruits**, Springer, 2007, p.221–235.

OLIVEIRA, L. S.; DIAS, P. C.; BRONDANI, G. E. Micropropagação de espécies florestais brasileiras. **Pesquisa Florestal Brasileira**, 33(76):439-453, 2013.

OLIVEIRA, T. R. et al. Light spectra affect the *in vitro* shoot development of *Cedrela fissilis* Vell. (Meliaceae) by changing the protein profile and polyamine contents. **Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics**, 1868(12), 2020.

PELEGRINI, L. L. et al. Micropropagation of *Ocotea porosa* (Nees e Martius) Barroso. **African Journal of Biotechnology Nairobi**, 10(9):1527-1523, 2011.

PELLEGRINESCHI, A. *In vitro* plant regeneration via organogenesis of cowpea [*Vigna unguiculata* (L.) Walp.]. **Plant Cell Reports***,* 17:89-95, 1997.

PEREIRA, A. et al. Micropropagation of *Salix humboldtiana* Hild. **Revista Brasileira de Plantas Medicinais**, 2(2):17-21, 2000.

PIERIK, R. L. M. **Cultivo *in vitro* de las plantas superiores***.* Madrid: Mundi-Prensa, 1990, 326p.

RIBEIRO, J. M. et al. Uso da rapadura como meio nutritivo para cultivo *in vitro* de bananeira cv. Maçã. **Revista Ceres**, 60(5):722-725, 2013.

RIZZINI, C. T.; MORS, W. B. **Botânica Econômica Brasileira**. São Paulo: Ed. EPU. 1976, 207p.

ROCHA, M. A. C. et al. Enraizamento *in vitro* e aclimatização de genótipos de jenipapeiro (*Genipa americana* L.). **Revista Brasileira de Fruticultura**, 30(3):769-774, 2008.

RUIZ-FILHO, R. R. et al. Fungos associados às sementes de cedro. **Summa Phytopathologica**, 30(4):494-496, 2004.

SANTOS, B. R. et al. Micropropagação de pequizeiro (*Caryocar brasiliense* Camb.). **Revista Brasileira de Fruticultura**, 28(2):293-296, 2006.

SCHENK, R. U.; HILDEBRANDT, A. C. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. **Canadian Journal of Botany**, 50:199-204, 1972.

SCHWEEN, G.; SCHWENKEL, H. G. Effect of genotype on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in greenhouse of *Primula* ssp. **Plant Cell, Tissue and Organ Culture**, 72:53-61, 2003.

SILVA, A. L. L. et al. Pré-aclimatização e aclimatização em cultivo hidropônico de plantas micropropagadas de *Eucalyptus saligna* Sm. **Revista Acadêmica: Ciências Agrárias e Ambientais**, 9(2):179-184, 2011.

SILVA, E. R. et al. *In vitro* germination of *Melanoxylon brauna* SCHOTT. and evaluation of the toxicity of disinfecting agents in the *Lactuca sativa* L. model plant. **Cerne**, 25(4):375-385, 2019.

SOS MATA ATLÂNTICA FOUNDATION. **Relatório Anual de Atividades SOS Mata Atlântica – Edição 2014**. Available in: <https://www.sosma.org.br/link/relatorio_anual_sosma_2014/index.html>. Access in: May, 08, 2021.

STEFANO, M. V.; CALAZANS, L. S. B.; SAKURAGUI, C. M. Meliaceae. In: **Lista de Espécies da Flora do Brasil**. Jardim Botânico do Rio de Janeiro. 2015. Available in: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB9990>. Access in: January, 16, 2019.

TIBOLA, C. T. et al. Diferentes meios de cultivo no enraizamento *in vitro* de porta-enxertos de *Prunus* sp. **Revista Brasileira de Agrociência**, 10(2):191-195, 2004.

UNITED NATIONS FRAMEWORK CONVENTION ON CLIMATE CHANGE - UNFCCC. NDC Registry. **Intended Nationally Determined Contribution (NDC) - Federative Republic of Brazil**. 2016. Available in: <https://www4.unfccc.int/sites/ndcstaging/PublishedDocuments/Brazil%20First/BRAZIL%20iNDC%20english%20FINAL.pdf>. Access in: May, 08, 2021. VILA, S. et al. Somatic embryogenesis and plant regeneration in *Cedrela fissilis*. **Biologia Plantarum**, 53(2): 383-386, 2009.

WATANABE, K. N.; RAMAN, K. V. Plant biotechnology and plant genetic resources: A global perspective. In: WATANABE, KN; PEHU, E. **Plant biotechnology and plant genetic resources for sustainability and productivity**. Austin: Academic Press, 1997, p.1-13.

WHITE, P. R. **The Cultivation of Animal and Plant Cells**, 2nd edn). New York: Ronald Press, 1963, p.57-63.