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Vol. 7(1), pp. 8-15, January, 2015
DOI: 10.5897/JHF2014.0367
Article Number: B77B67549548
ISSN 2006-9782
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Journal of Horticulture and Forestry

Full Length Research Paper

Propagation of Cabralea canjerana by mini-cutting

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Received 27 August, 2014, Accepted 16 October, 2014

Canjerana (*Cabralea canjerana* (Vell.) Martius) is a tree species indigenous to Brazil that when grown and managed in plantation systems are of great ecological and economic importance. Due to the difficulty of producing seminal seedlings, we examine the possibility of vegetative propagation by evaluating the rooting potential of canjerana mini-cuttings with different concentrations of indolbutyric acid (IBA) and substrate combinations. Mini-cuttings were treated with 2000 mg/L of IBA and planted in commercial substrate; coarse sand; carbonized rice husks; and a combination of the two. Apical and nodal mini-cuttings were treated with 0, 1000, 2000 and 3000 mg/L of IBA and planted in a combination of commercial substrate, coarse sand and carbonized rice husks. A mini-clonal hedge was formed with three clones of canjerana to evaluate mini-stump productivity and mini-cutting rooting. The combination of commercial substrate, coarse sand and carbonized rice husks maximized mini-cuttings rooting. Nodal mini-cuttings had higher rooting capability than apical ones. The application of 3000 mg/L of IBA improved rooting differentiation and growth of canjerana mini-cuttings. Canjerana clones differ in rooting capability and survival rates in vegetative propagation systems, but the use of a mini-cutting propagation system is a feasible production technique for this important species.

Key words: Vegetative propagation, miniclonal hedge, mini-cutting rooting, indolbutyric acid.

INTRODUCTION

Cabralea canjerana (Vell.) Martius, known as canjerana in southern Brazil, is a native tree species that belongs to the Meliaceae family. Canjerana is a common species in some Atlantic Forest fragments, and it is very important for forest regeneration programs in degraded areas (Nobrega et al., 2008). The wood is considered to be one of the most valuable, with excellent quality characteristics and high resistance to the attack of xylophage insects. The stem bark can be used for the extraction of a commercially important red dye. Both the stem bark and the roots have medicinal properties and can be used as a purgative, febrifuge, abortifacient, antidyspeptic, astringent and emetic (Carvalho, 2006). Extracts from leaves and seeds can affect the development of *Ascia monuste orseis* larvae, reducing leaf consumption in cabbage (Mata and Lomonaco, 2013).

The seeds of canjerana present recalcitrant storage behavior, with a drastic reduction in germination after 15 days of beneficiation (Grunennvaldt et al., 2014), which is an obstacle for the production of seminal seedlings. Vegetative propagation enables the production of plantlets in any season and the establishment of specific

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and favorable combinations of important characters, as well as the possibility of fixing superior genetic interactions and high levels of heterozygosity (Zobel, 1993; Gaspar et al., 2005). As canjerana provides wood and other products, the development of efficient clonal propagation techniques for superior genotypes will increase management and productivity of commercial plantations, thereby contributing to local economies.

The use of mini-cuttings is one of the most recent techniques for vegetative propagation. The mini-cutting technique can enhance propagation efficiency and was developed to overcome the rooting problems of some adult plants of the genus *Eucalyptus* (Wendling et al., 2003). Mini-cuttings from rejuvenated propagules are used for regeneration and mass production of woody species plantlets (Hartmann et al., 2011). In some clones of *Eucalyptus* the mini-cuttings from rejuvenated plant material develop a better root system than is found in mini-cuttings from non-rejuvenated tissue (Wendling and Xavier, 2005). Research results with other tree species indicate the viability of using rooted mini-cuttings for commercial propagation (Dumroese et al., 2003; Xavier et al., 2003; Wendling et al., 2007; Lima et al., 2009; Silva et al., 2010; Dias et al., 2012).

In the plantlet production from mini-cuttings, the substrate plays a fundamental role in root initiation and growth. It keeps the mini-cuttings in an upright position, provides a matrix for needed water and nutrients, and improves root differentiation and growth. Because of these specific but multifaceted requirements, the best substrate is usually composed of a variety of different materials that fulfill these physical functions and facilitate the hormonal stimulation of the desired growth responses, as well as providing availability of needed assimilates for rooting and plant health (Fonteno et al., 1981; Smart et al., 2003). Frequently in forest species, the inclusion of exogenous indolbutyric acid (IBA) has been shown to improve root differentiation (Wise et al., 1985; Rosier et al., 2004; San José et al., 2012). However, mini-cuttings of some woody species have been shown to root even without the application of IBA (Silva et al., 2010; Ferreira et al., 2010).

The choice of the substrate for the production of plantlets should take into consideration physical factors, such as texture and density, which interfere with aeration, water retention capacity and aggregation of the substrate (Wendling and Gatto, 2002). A good substrate must provide sufficient porosity to allow good aeration and have a high water retention capacity (Hartmann et al., 2011). Recommendations in the literature vary from an addition of 20 to 40% or a reduction of 60 to 80% of porous material (carbonized rice husks, coconut fiber, pine needles, compost, etc.) for the substrate composition for different species and propagation conditions (Wendling and Gatto, 2002). Substrates with air-filled pore space of 30%, with total porosity of 85% and water availability between 24 and 40% were considered as ideal conditions for seedling production (Schimitz et al., 2002), but no such information was found for mini-cuttings rooting. The three individual substrates, carbonized rice husks, commercial substrate, and coarse sand, and their combinations exhibit different physical properties from those considered ideal by Schimitz et al. (2002) for seedlings production. Carbonized rice husks increase the total porosity and air-filled pore space in the combination of substrates, but not to the optimum level suggested by Schimitz et al. (2002). This probable explains why the best substrate is usually obtained by using a mixture of components to ensure nutritional balance and diverse microbial flora (Wendling and Gatto, 2002). Given the ecological and economic importance of canjerana and the difficulty of producing seminal seedlings, this study was intended to examine the possibility of vegetative propagation by mini-cuttings. The objective was to evaluate the rooting potential of canjerana mini-cuttings with different concentrations of IBA and substrate combinations.

MATERIALS AND METHODS

The study was carried out in an acclimatized greenhouse of polycarbonate 10 mm panels with maximum temperature set to 32°C. For the experiments, one-year-old seedlings of canjerana were placed in plastic pots containing 300 cm^3 of a commercial substrate (organic pine bark base) and submitted to drastic pruning (coppice) to produce the sprouts that were used for mini-cutting preparation after 60 days. The coppice seedlings were manually irrigated twice a week with a nutrient fertilizer solution with 50% of the salt concentrations as described by Wendling et al. (2007). The mini-cuttings were grown in a vertical position in polyethylene trays (55 × 34 × 15 cm) containing the substrate and maintained in a greenhouse with a system of intermittent misting, mean air temperature of 25°C, and relative humidity of approximately 80%. The high relative humidity was maintained by nebulizers, triggered by a timer. After evaluating rooting and survival in a mist chamber, the rooted mini-cuttings were acclimatized in a greenhouse for 30 days and used for the formation of the mini-clonal hedge.

The first experiment evaluated the effect of the substrate on mini-cutting rooting and survival. The bases of single bud mini-cuttings, 1.5 to 2.0 cm long containing a half leaflet, were immersed for 10 s in a 50% water/ethanol solution containing 2000 mg/L of IBA and placed in one of the four substrates: commercial (organic pine bark base); coarse sand; carbonized rice husks; and the combination of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks. Samples of these substrates were collected, dried at 65°C for 48 h and submitted for physical analysis (Fermino, 2003). Carbonized rice husks and the mixture of commercial substrate, coarse sand and carbonized rice husks were the substrates with air-filled pore space that came closer to the optimal value of 30% (Table 1). After 60 days of cultivation, mini-cuttings were evaluated for rooting and survival and the number and total length of roots (cm). The experiment was a complete random design with five replicates of four mini-cuttings.

The second experiment evaluated the type of mini-cuttings (apical and nodal) of 1.5 to 2.0 cm in length containing a half leaflet. The bases of mini-cuttings were immersed for 10 s in a 50% water/ethanol solution containing either 0, 1000, 2000 or 3000 mg/L of IBA. The control treatment consisted of a 50% water/ethanol solution. A mixture of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks was used as substrate. The rooting and survival of the shoots and the number

Table 1. Physical properties of the substrates used for the evaluation of the rooting capacity of canjerana.

Substrates[1]	Dry density (kg/m^3)	Total porosity (%)	Aeration space in saturated substrate (%)	Water availability (%)
Comercial	335	50	15.4	5.4
Coarse Sand	1505	27	12.6	11.5
CRH	167	41	31.4	2.8
Comercial+CS+CRH	56	43	23.9	4.4

[1]Commercial: organic pine bark base; CS: coarse sand; CRH: carbonized rice husks.

Table 2. Percentage survival and rooting and number and length of roots of mini-cuttings of canjerana treated with 2,000 mg/L of indolbutyric acid and grown in different substrates after 60 days of cultivation.

Substrates[1]	Survival (%)	Rooting (%)	Number of roots	Length of roots (cm)
Comercial	70.0^{b2}	15.0^{b}	1.8^{a}	3.3^{a}
Coarse sand	70.0^{b}	45.0^{b}	1.8^{a}	2.5^{a}
CRH	85.0^{b}	30.0^{b}	1.8^{a}	2.7^{a}
Comercial+CS+CRH	100.0^{a}	75.0^{a}	1.7^{a}	3.0^{a}
Mean	81.2	41.2	1.8	2.9
CV (%)	14.1	39.4	14.4	8.6

[1] Commercial: organic pine bark base; CS: coarse sand; CRH: carbonized rice husks. [2] Means values followed by the same letter in a column are not significantly different by the Tukey's test at the probability of 5%.

and total length of roots (cm) per mini-cutting were evaluated after 60 days of cultivation. The experiment was a 2 x 4 factorial (apical and nodal mini-cuttings by IBA concentrations) in a complete random design with five replicates of four mini-cuttings.

The third experiment evaluated the productivity of individual mini-stumps and rooting of mini-cuttings of three clones of canjerana. The mini-clonal hedge was established in a soilless system subirrigated with nutrient solution (Bandinelli et al., 2013). Twelve rooted mini-cuttings (complete plantlets) per single stock plant of canjerana were planted at 10 x 10 cm spacing in one tray to form the mini-clonal hedge and given a 15 min daily irrigation with a nutrient solution at 50% concentration of salts as described by Wendling et al. (2007). The pH of the nutrient solution was maintained between 5.5 and 6.0 and an electrical conductivity in 1.5 dS m^{-1}. The sprouts of three consecutive harvesting times of the three clones were used to prepare mini-cuttings of 1.5 to 2.0 cm in length containing a half leaflet. The mini-cuttings were treated with 3000 mg/L of IBA solution for 10 s and planted in a combination of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks. The number of mini-cuttings per mini-stump was recorded and the percentage of survival and rooting were evaluated at 60 days after planting. The experiment was a complete random design with eight replicates of six mini-cuttings.

Data were submitted to analysis of variance and for those variables with significant differences ($p \leq 0.05$), treatment means were compared by Tukey test or polynomial regression, as appropriate. For purposes of analysis, percentage data were transformed to arcsin$\sqrt{x/100}$ and counting data to $\sqrt{x+0.5}$ to attend the statistical presuppositions. All analysis well done with the ESTAT (UNESP - Jaboticabal) program.

RESULTS AND DISCUSSION

The different substrates affected the rooting and survival percentages of canjerana mini-cuttings ($p \leq 0.05$). The combination of commercial substrate, coarse sand and carbonized rice husks resulted in the highest percentage of mini-cutting rooting (Table 2). The number and length of roots per mini-cutting did not differ significantly across substrates. On average, rooted mini-cuttings produced 1.8 roots with an average total length of 2.9 cm. In the experiment using individual substrates (soil, sand, carbonized rice husks, peat, decomposed residue of black wattle bark) and their combinations, only the sand showed a value of available water within the ideal range for its use as a substrate for seedlings production, which implies that this material can ensure high water availability (Schimitz et al., 2002). The increased survival in combined substrate (Table 2) may reflect the effect of the coarse sand resulting in increased water availability to the mini-cuttings of canjerana. For *Maytenus ilicifolia* (Mart. ex Reissek) a higher rooting percentage (94.3%) was obtained with mini-cutting cultivation in pure commercial substrate (Lima et al., 2009). Rooting of *Calophyllum brasiliense* (Camb.) mini-cuttings was lower in commercial substrates (bark composted pine) and carbonized rice husks when compared with vermiculite (Silva et al., 2010). Clearly then, the optimal substrate varies according to species and conditions of rooting, and substrate evaluation is essential in optimizing rooting protocols given their determinant role in defining the range of root induction. Mini-cuttings from nodal segments showed a higher percentage of rooting and an increased number and length of roots in comparison to

Table 3. Percentage of survival and rooting and number and length of roots of apical and nodal canjerana mini-cuttings after 60 days of cultivation.

Mini-cuttings	Survival (%)	Rooting (%)	Number of roots	Total length of roots (cm)
Nodal	80.0^{a1}	50.0^{a}	2.2^{a}	6.3^{a}
Apical	74.0^{a}	17.0^{b}	1.4^{b}	3.0^{b}
Mean	77.0	34.0	1.8	4.6
F value^{2}	0.2^{ns}	5.3**	4.5**	36.6**
CV (%)	22.7	57.1	21.0	13.6

[1] Means values followed by different letters in a column are significantly different by the F test at the indicated probability. [2] (^{ns}) no significant, (*) significant at 5% of probability, and (**) significant at 1% of probability.

Table 4. Percentage survival and rooting and number and length of roots of mini-cuttings of canjerana treated or untreated with indolbutyric acid (IBA) after 60 days of cultivation.

Mini-cuttings	Surviv^{a}l (%)	Rooting (%)	Number of roots	Tot^{a}l length of roots (cm)
Treated with IBA	79.3^{a1}	43.3^{a}	2.0^{a}	5.2^{a}
Untreated	70.0^{a}	5.0^{b}	1.1^{b}	2.6^{b}
Mean	74.6	24.1	1.5	3.9
F value^{2}	1.3^{ns}	12.9**	14.8**	70.0**
CV (%)	17.8	52.1	13.6	10.5

[1] Means values followed by different letters in a column are significantly different by the F test at the indicated probability.
[2] (^{ns}) no significant, (*) significant at 5% of probability, and (**) significant at 1% of probability.

the apical ones (Table 3). The increment in rooting percentage of nodal segments was 2.9 folds. No significant difference in survival was observed between apical and nodal mini-cuttings. Interestingly, there were no significant differences ($p \geq 0.05$) in the responses of nodal versus apical explants to the IBA treatments, so data from the apical and nodal mini-cuttings were combined in Table 4. Given the lack of IBA treatment effects in this experiment, the differences found between nodal and apical mini-cuttings may be associated with the levels of carbohydrates, amino acids and other substances that promote adventitious rooting of certain tissues of the mini-cuttings, as noted by Hartmann et al. (2011). Thus, the mini-cuttings from nodal segments may have more appropriate levels of reserves than the apical ones, which would improve rooting capability. In addition, cells that have hormones or other compounds that confer an endogenous potential for root formation, such as auxin, quickly react to specific stimuli, like light and temperature (Sorin et al., 2005). It is possible that mini-cuttings from nodal segments have a better competence for rooting, but it does not affect mini-cutting survival.

The mini-stumps of canjerana had adequate nutritional level and the mini-cuttings for comparisons between clones were taken from the same sprouting period, with differences thus being an indication of existing genetic variation between canjerana clones, as expected. These are the first results of canjerana vegetative propagation by mini-cuttings and clearly indicate the effect of nodal and apical mini-cuttings on root initiation and growth. These differences are very important in developing a system for mass production of plantlets, because of economic and labor usage issues. It is possible that these differences are minimized with the advancing age of the shoots and the size of the mini-cuttings (greater than 2.0 cm for example), which should be the aim of future studies.

In other species, such as *Calophyllum brasiliense* (Camb.), apical and nodal mini-cuttings did not differ for rooting (Silva et al., 2010). These results were attributed to the good nutritional status of stock plants of the mini-clonal hedge, as well as to the physiological age of the two types of mini-cuttings, which makes them similar in the degree of juvenility and tissue lignification. In a study with mini-cuttings of *Anadenanthera macrocarpa* (Benth) Brenan, apical mini-cuttings were more responsive to rooting than nodal ones (Dias et al., 2012), being attributed to the greater degree of juvenility and less lignification of apical tissues (Xavier et al., 2003). Furthermore, apical mini-cuttings have a higher concentration of endogenous rooting promoters by virtue of their proximity to both the sites of auxin synthesis and to less differentiated tissues, potentially resulting in increased dedifferentiation of cells to their meristematic condition, which is essential for root initiation (Gehlot et al., 2014).

The application of IBA increased ($p \leq 0.05$) the percentage of rooting and the length and number of roots per canjerana mini-cutting (Table 4). The application of IBA did not significantly affect the survival of the mini-

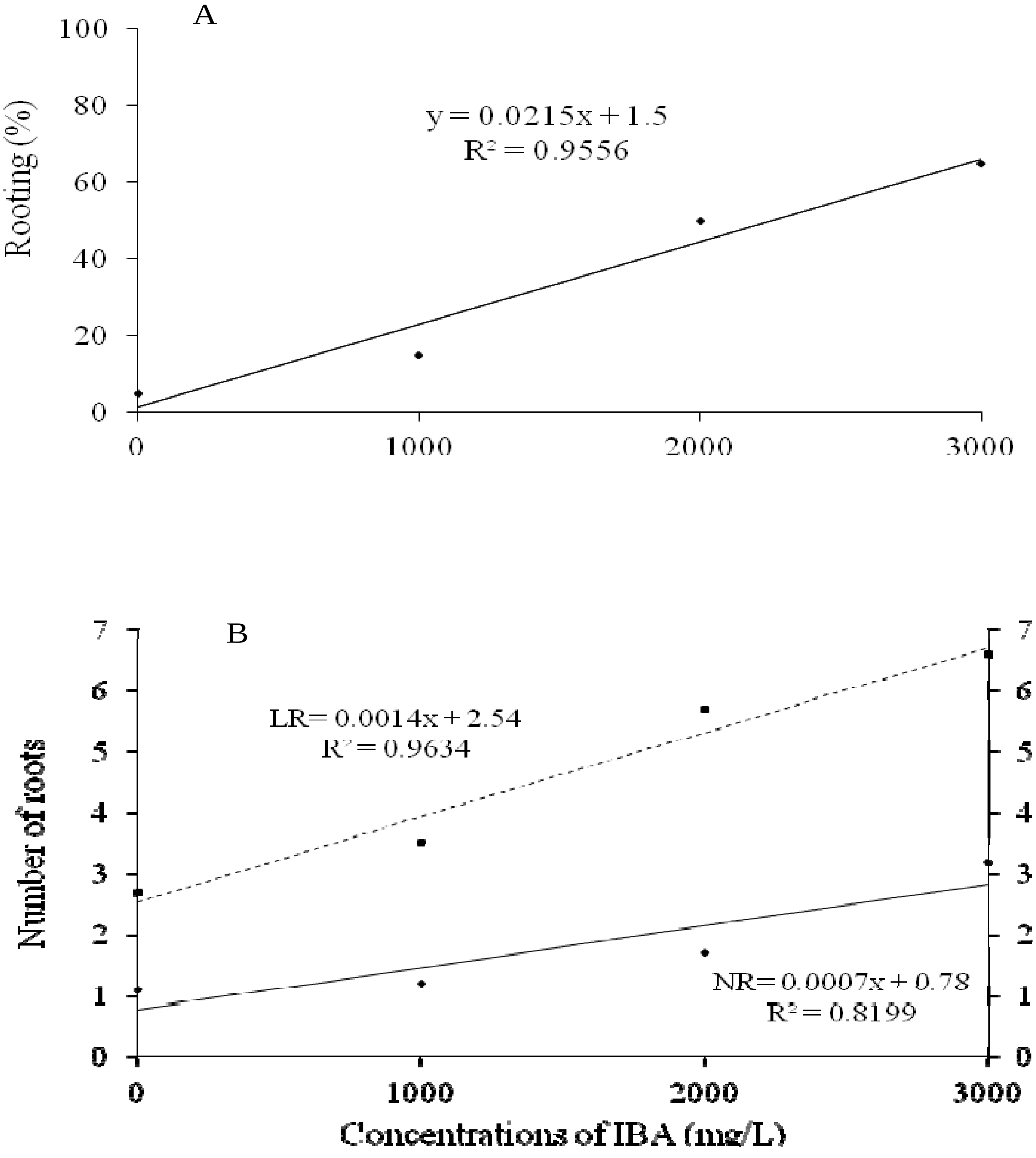

Figure 1. Rooting percent (A) and number (NR) and length of roots (LR) (cm) (B) of mini-cuttings of canjerana treated with different concentrations of indolbutyric acid (IBA) after 60 days of cultivation.

cuttings. Increasing the concentration of the IBA treatment improved the percentage of rooting and increased both the number and length of roots (Figure 1). At 60 days, canjerana mini-cuttings treated with IBA showed a higher percentage of survival and rooting, as well as increased root growth compared with mini-cuttings with no IBA treatment (Table 4). Auxin application enhances root formation in species with low rooting response, as governed by either genotype or physiological stage (Xavier et al., 2003). It is possible that low rooting response is caused by low content of endogenous auxin, which often requires the exogenous application of growth regulators to establish the competence and determination of target cells (Taiz and Zeiger, 2008). Therefore, canjerana fits in this group of species, because the application of IBA increased both

Table 5. The average number of mini-cuttings (NM) and percentages of survival (S) and rooting (R) of mini-cuttings of canjerana harvested at three different harvested dates from mini-stumps formed from mini-cuttings that were rooted in a soilless system subirrigated with nutrient solution.

Clones	Harvest I			Harvest II			Harvest III		
	NM	S (%)	R (%)	NM	S (%)	R (%)	NM	S (%)	R (%)
SM1	2.5^{a1}	84^{c}	53^{b}	2.7^{a}	73^{c}	69^{a}	2.8^{a}	72^{a}	72^{b}
SM3	2.4^{a}	98^{a}	50^{b}	2.5^{a}	79^{b}	51^{b}	2.6^{a}	55^{b}	52^{b}
SM13	1.8^{a}	93^{b}	67^{a}	2.9^{a}	90^{a}	68^{a}	3.0^{a}	81^{a}	80^{a}
Mean	2.2	92	57	2.7	81	63	2.8	69	68
CV (%)	12.04	3.69	3.84	11.05	3.47	4.37	10.52	3.23	4.99

[1] Means values followed by the same letter in a column are not significantly different by the Tukey's test at the probability of 5%.

rooting percentage and the number and length of the new roots (Table 4 and Figure 1), which explains the higher rooting of canjerana mini-cuttings treated with IBA.

The observed differences in average root length may be associated with the increase in rooting competence promoted by IBA application that resulted in a rapid differentiation of the tissues for the formation of adventitious roots in canjerana mini-cuttings. In this study, only the nodal mini-cuttings treated with 2000 or 3000 mg/L of IBA had already developed adventitious roots at 30 days after cultivation (data not shown). The use of IBA resulted in an increased percentage of rooting and both the number and length of roots in the canjerana mini-cuttings (Figure 1). Also, the use of IBA favored rooting and did not affect the survival of mini-cuttings (Table 4), indicating that there was no phytotoxic effect in any of the tested concentrations (Figure 1). Mini-cuttings responded to an increase in IBA concentration up to 3000 mg/L, which can be considered a high concentration for mini-cuttings. At that concentration, 65% of mini-cuttings rooted, with an average of 3.2 roots per mini-cutting which averaged 6.6 cm in length at 60 days following cultivation. This contrasts with some previous mini-cutting studies in which the use of IBA concentrations above 2000 mg/L had negative effects on rooting due to the high degree of juvenility of the new shoot (Titon et al., 2003). It is thought that this is due to the fact that the juvenile mini-cuttings already have tissues with an endogenous hormonal balance that is favorable for rooting, which then leads to no (Silva et al., 2010; Ferreira et al., 2010; Wendling et al., 2010) or even a negative response to exogenous application of IBA (Xavier et al., 2003).

The production of mini-cuttings per mini-stump did not differ among the clones of canjerana that were evaluated in this study. The lowest production of mini-cuttings per mini-stump was 1.8 in the first harvest period and the highest was 3.0 in the third harvest (Table 5). The survival of mini-stumps was 95% and did not differ among harvest dates or clones (data not shown). In mini-cuttings from the mini-clonal hedge grown in a soilless system using subirrigation with a nutrient solution, the average production of mini-cuttings per harvesting date was 2.2 for the first harvest period, 2.7 for the second harvest period, and 2.8 for the third harvest period. These values were higher than has been reported for *Cedrella fissilis* (Vell.) (Xavier et al., 2003) and lower than those observed in *Ilex paraguariensis* (St. Hil.) (Wendling et al., 2007). There were significant differences in rooting ($p \leq 0.05$) among clones and harvest dates. The rooting percentage was 50% or greater in all treatments and showed an increase during the successive harvest periods. Interestingly, survival percentage dropped as rooting percentage increased (Table 5). In a study with *Liquidambar styraciflua* (L.), the survival percentage of rooted minicuttings was influenced by relative humidity and sunlight during the acclimatization period in the greenhouse and outdoor conditions (Wendling et al., 2010). Therefore, increasing the harvesting period of canjerana mini-cuttings can enhance the productivity of mini-stumps, and the maintenance of mini-cuttings in the greenhouse conditions may increase survival and rooting, as mentioned by Wendling et al. (2010). The decline in survival was particularly evident in the clone SM3. This interplay between survival, rooting percentage, and clone indicates the need for tailoring production systems specifically for each clone that will be propagated.

The results of this study clearly show that vegetative propagation by mini-cuttings is a feasible alternative for the mass production of canjerana plantlets for commercial plantation establishment (Figure 2). Apical and nodal mini-cuttings with 1.5 to 2.0 cm in length containing a half leaflet may be planted in media consisting of the same proportions of commercial substrate, coarse sand and carbonized rice husks. Clearly, though, nodal mini-cuttings have a higher competence for rooting. Based on this work, mini-cuttings should be treated with 3000 mg/L of IBA for the greatest rooting efficiency. Given clonal differences in these studies, it is possible that mini-cuttings with different amounts of reserves and/or from other clones might have a greater competence for rooting or higher survival rates during the propagation process, which would facilitate the mass production of plantlets, possibly with lower

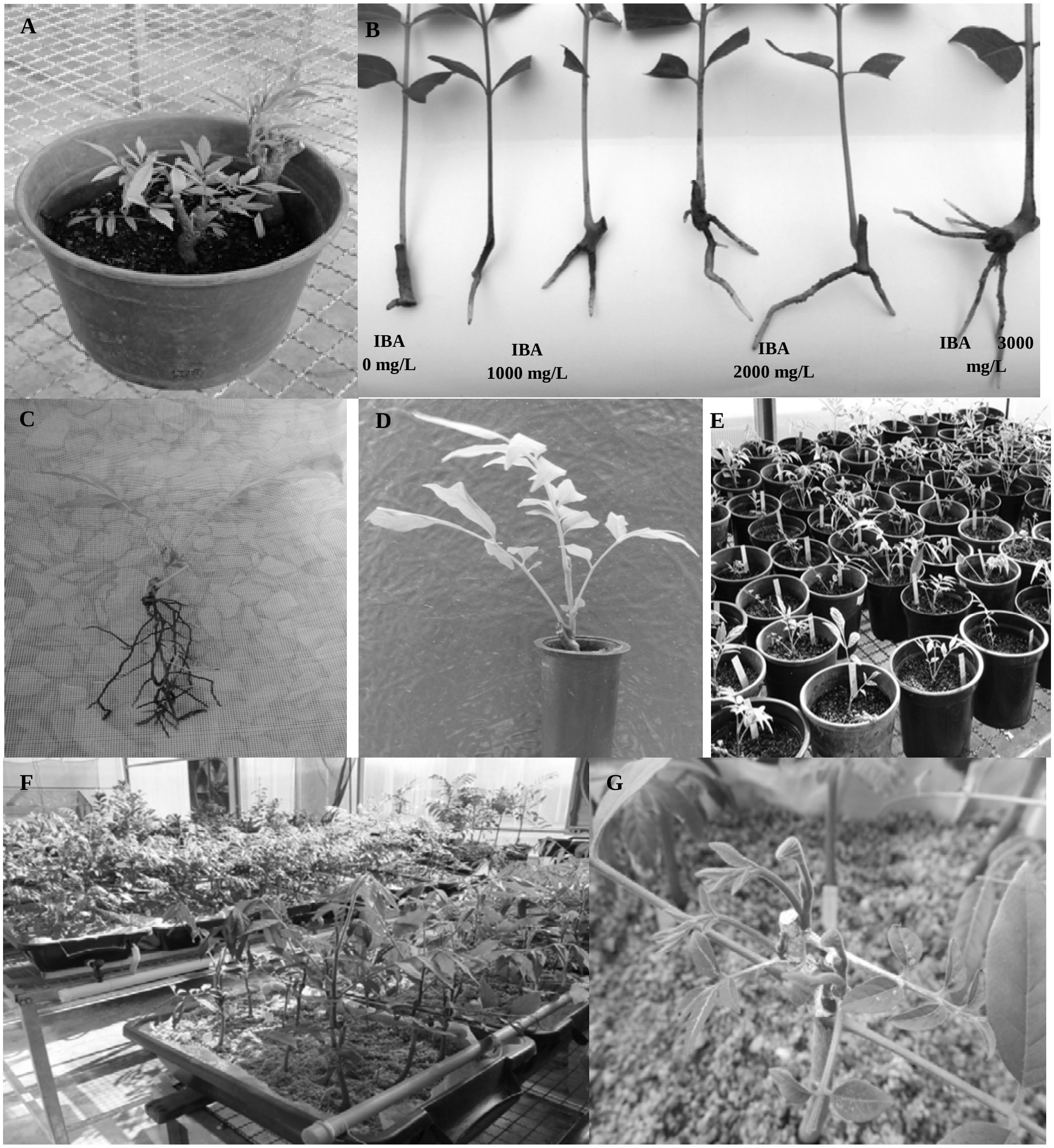

Figure 2. Shoots in mini-stump of canjerana (A), mini-cutting (nodal segment) rooted with different concentrations of indolbutyric acid (IBA) (B), seedling with four months of age (C), acclimatized seedling in tube (D), rooted mini-cuttings being acclimatized in pots (E), mini-clonal hedge in a soilless system (coarse sand as substrate) subirrigated with nutrient solution (F), and mini-stumps with shoots (G).

concentrations or even without the application of IBA. Once the rooted mini-cuttings are acclimatized in the greenhouse they may be transferred to either the mini-clonal hedge or to a full sun area for further acclimatization (Figure 2). The new plantlets can then be effectively planted in the field for growth and wood quality evaluations. This work shows that the use of mini-cuttings is a feasible technique for mass production of canjerana plantlets for plantation establishment from selected clones in order to enhance management and growth of this important species.

Conflict of Interest

The authors have not declared any conflict of interest.

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