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Vegetative rescue of *Melanopsidium nigrum* Colla via induction of adventitious roots

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A R T I C L E I N F O

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A B S T R A C T

Cloning *Melanopsidium nigrum* mature trees from detached older branches by cutting is an important tool to conserve the species as it is listed as vulnerable to extinction. We selected sixty-four mother trees, and ten to fifteen branches from each mother tree were used to make cuttings (n = 822) which were maintained in a greenhouse. Half of the cuttings were treated with indole-3-butyric acid (6000 mg.Kg^{-1}), applied as a powder conjugated with talc at the base of the cutting. At 120 days after cutting, some cuttings were alive and rooted, corresponding to a production index of 9.5% and 7.3% for cuttings from the control treatment and treated with auxin, respectively. This work is pioneer in studying the adventitious rooting of *M. nigrum*, and has demonstrated the possibility of propagation of this species through this technique.

The *restinga* is one of the ecosystems of the Atlantic Forest in Eastern Brazil, composed of plant communities that receive great influence from the coastal environment, such as strong winds, salt-spray, and high solar radiation. Its vegetation develops on Quaternary marine sandy sediment deposits (Veloso et al., 1991), with a low concentration of mineral nutrients, due to high leaching (Guedes et al., 2006). *Restingas* are important for biodiversity and coastal dynamics, with herbaceous, shrubs, and forests vegetation types (Thomazi et al., 2013). Over time, due to *habitat* degradation and fragmentation, large losses of biodiversity resulting from human activities are observed (Cabral and Fiszon, 2004).

One of the species of this ecosystems *Melanopsidium nigrum* Colla (Rubiaceae), which is found in the *restingas* and ombrophilous forests of the southeastern region, in the states of Espírito Santo, Rio de Janeiro and Bahia and the *cerrado* in Minas Gerais (Zappi et al., 2009; Zappi, 2012). In the *restinga*, *M. nigrum* occurs mainly in forest and thicket typologies, in areas not subject to flooding. They are shrubby or arboreal, dioecious, ranging from 1.5 to 5 m of height in the *restingas*, reaching 10 m in forest ecosystems, with cylindrical, furrowed branches, often erect. The flowering period of *M. nigrum* occurs from July to November, and fruiting occurs between February and April (Delprete, 2000). Currently, this species is threatened with extinction, mainly due to habitat loss, and classified as vulnerable – VU, according to International Union for Conservation of Nature (IUCN) criteria (Martinelli and Moraes, 2013).

Due to the fragility of this species, Brazilian environmental agencies require the transplantation of individuals found in areas of suppression of vegetation for the installation of enterprises. However, there is a high mortality rate after transplanting adult plants. In this way, vegetative propagation becomes an important strategy to allow additional success for the compensatory rescue and the maintenance of its populations genetic diversity.

Adventitious root formation is a key step in vegetative propagation by stem cuttings, and has been exploited in horticulture, agriculture, and forestry (Bonga, 2016). Although endogenous and environmental factors interfere in the success of root formation, this technique is one of the

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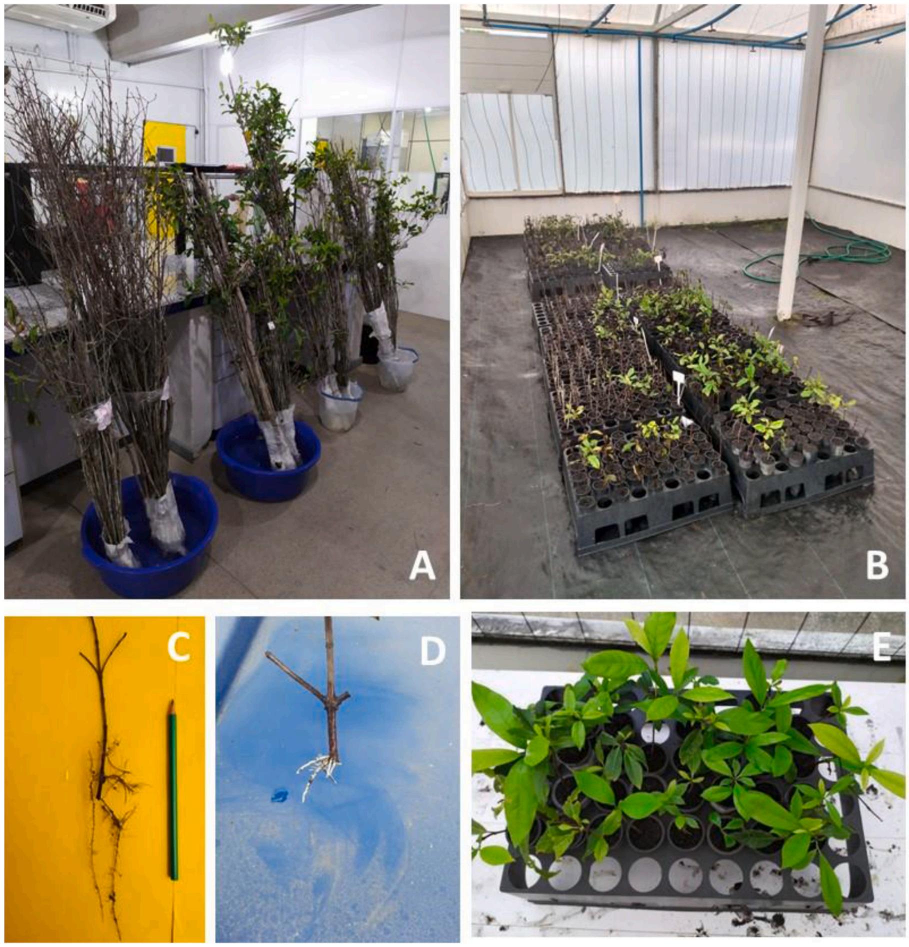

Fig. 1. A: Arrival of the collected branches in the Laboratory of Plant Physiology/UENF; B: Trays with the seedlings in the greenhouse (nebulization/rooting sector); C: Control plant root (roots originating from several points on the cutting); D: Root of a cutting that has been treated with IBA; E: Seedlings formed at 120 days after staking.

most important for clonal propagation. The use of auxins to stimulate rooting in difficult-to-root species is trivial but their use in *M. nigrum* is not known.

In October 2021, an area of *restinga* (4552 ha), located in the municipality of São João da Barra (21° 38′ 56″ S; 41° 3′ 9″ W, State of Rio de Janeiro, Brazil), received authorization to remove vegetation from the competent environmental agency for the implementation of a thermoelectric park. All threatened flora species, including *M. nigrum*, were removed from the area and replanted in the Fazenda Caruara Natural Heritage Private Reserve (RPPN Caruara), the largest private Brazilian reserve dedicated to the preservation of the *restinga* ecosystem, with approximately 4000 ha.

Thus, to preserve the genetic material of each of the 64 individuals of *M. nigrum* directly affected, they were transplanted in the RPPN Caruara in a place with characteristics similar to their area of origin. At the time of transplanting, branches (100–180 cm) were collected from these individuals to assess the possibility of multiplication by propagation from cuttings. As the plants were naturally distributed in the suppressed area, there is no information about the chronological age of the matrices. Regarding the presence of leaves, flowers, and fruits, the material collected showed heterogeneity among individuals (Fig. 1A). The plants presented variations in the thickness and flexibility of the branches.

After collection, the branches were kept in the shade, with the bases immersed in water, until transport to the university laboratory, located 45 km from the suppression area. The collection of branches took place between October 2nd and 15th, 2021. The branches were sectioned (20 cm) and the cuttings had a diameter at the base of 3–4 mm. Cuttings were obtained from segments of the base, the middle region and the upper part of the entire branches. The number of cuttings per matrix varied according to plant material availability, always multiples of 5 or 6. Half of the cuttings (n = 411) were treated with 3-indole butyric acid (IBA; 6000 mg.Kg^{-1}), applied as a powder conjugated with talc at the base of the cutting. The other half received no treatment. The cuttings were inserted into plastic tubes (280 cm^3) filled with a commercial substrate made of pine bark, coconut fiber, peat fiber, and vermiculite (Basaplant Florestal®). The trays with the plastic tubes were allocated in a greenhouse under intermittent nebulization (every 15 min for 10 s; mean temperature and humidity of 26 °C and 76%, respectively) for 60 days. Data for survival percentage (%) was submitted to descriptive analysis and comparison by Confidence Interval ($P < 0.05$) by Student's T-test (Rmisc package, Hope, 2013) through R software (R Core team, 2019).

At 60 days after staking (DAS), the cuttings were removed from the mist chamber and kept in the greenhouse with controlled irrigation and 70% shading of solar radiation interception.

The survival percentage of cuttings was evaluated at 60 DAS. The presence or absence of leaves in all cuttings (discarded cuttings and cuttings that were kept for evaluation) was verified. At 120 DAS, the production index was evaluated through the number of rooted cuttings concerning the total number of cuttings that were placed to root.

At 60 DAS, the survival percentage was 28.81% for the untreated cuttings and 26.5% for the cuttings treated with IBA, which showed that the use of IBA did not influence the survival of the cuttings. At 60 DAS, all live cuttings had remaining leaves and/or newly formed leaves. At

Table 1
Survival Rate at 60 and 120 days after staking (DAS) considering each individual matrix (n = 5; 6; 10 or 12 repetitions for each treatment). Control plants (without plant growth regulator treatment); IBA (clonal seedlings originated from cuttings treated with indole 3- butyric acid, 6000 mg/Kg^{-1}). The dots represent zero (0%).

Matrix Number	Survival (60 DAS) Treatments		Survival (120 DAS) Treatments		Matrix Number	Survival (60 DAS) Treatments		Survival (120 DAS) Treatments		Matrix Number	Survival (60 DAS) Treatments		Survival (120 DAS) Treatments	
	Control	IBA	Control	IBA		Control	IBA	Control	IBA		Control	IBA	Control	IBA
905	100%	60%	100%	–	**890**	40%	10%	–	–	**885**	10%	20%	–	–
910	100%	40%	80%	–	**626**	40%	–	40%	–	**913**	–	100%	–	80%
934	100%	33,3%	–	–	**625**	40%	–	10%	–	**908**	–	100%	–	–
915	100%	20%	20%	–	**899**	40%	–	–	–	**937**	–	40%	–	40%
907	80%	60%	60%	–	**933**	33,3%	67%	–	67%	**918**	–	33,3%	–	–
924	80%	60%	–	40%	**936**	33.3%	–	33.30%	–	**927**	–	33,3%	–	–
914	80%	40%	60%	–	**606**	30%	60%	20%	40%	**929**	–	33,3%	–	–
935	60%	80%	–	40%	**633**	30%	10%	–	–	**620**	–	30%	–	–
912	60%	80%	40%	20%	**634**	30%	10%	–	–	**923**	–	25%	–	–
909	60%	80%	20%	20%	**608**	30%	50%	–	20%	**921**	–	20%	–	–
891	60%	60%	40%	20%	**898**	30%	–	–	–	**896**	–	10%	–	10%
940	60%	20%	20%	–	**930**	20%	60%	–	–	**621**	–	10%	–	–
607	60%	10%	60%	10%	**897**	20%	40%	20%	–	**886**	–	10%	–	–
893	60%	–	–	–	**637**	20%	40%	–	20%	**628**	–	–	–	–
938	40%	60%	–	40%	**892**	20%	40%	–	–	**644**	–	–	–	–
889	40%	60%	–	–	**629**	20%	–	–	–	**887**	–	–	–	–
911	40%	40%	–	60%	**895**	20%	–	–	–	**888**	–	–	–	–
604	40%	30%	10%	–	**919**	20%	–	–	–	**916**	–	–	–	–
609	40%	26.7%	–	–	**939**	20%	–	–	–	**917**	–	–	–	–
928	40%	20%	–	–	**894**	10%	50%	10%	10%	**920**	–	–	–	–
617	40%	10%	20%	–	**601**	10%	20%	–	–	**922**	–	–	–	–
										925	–	–	–	–

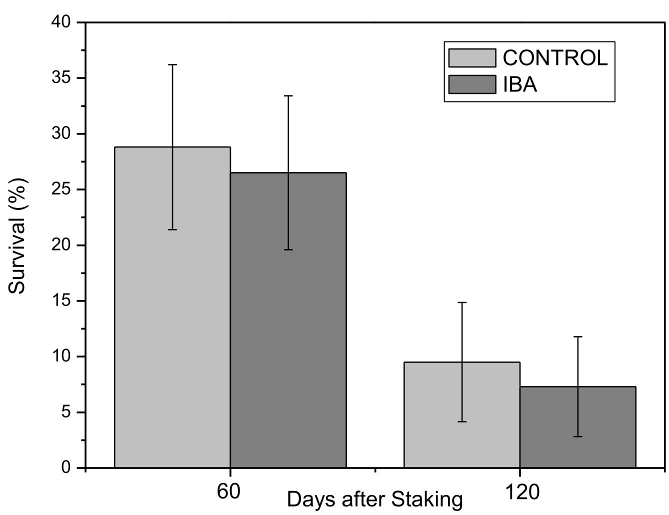

Fig. 2. Survival (%) at 60 and 120 days after staking (DAS). Control plants (without plant growth regulator treatment); IBA (seedlings originated from cuttings treated with indole 3- butyric acid, 6000 mg/Kg^{-1}). The data are expressed as the mean with confidence interval using Student's t-test ($P < 0.05$).

this time, cuttings without leaves, due to the absence of these organs in their preparation or due to the abscission process, were discarded because they did not have roots.

Of the 64 matrices tested, cuttings from nine individuals did not survive until 60 DAS (Table 1), and 33 individuals had a variable number of live cuttings. Cuttings from ten matrices only showed live cuttings in the control treatment, and twelve individuals only showed survival of IBA-treated cuttings (Table 1).

At 120 DAS, only 69 seedlings from 28 individuals were alive and rooted, corresponding to a Production Index of 9.5% and 7.3% for cuttings from the control treatment and treated with IBA, respectively (Fig. 2). Thus, it is possible to infer that the root induction capacity is more related to the intrinsic characteristics of the individual (phenology, nutritional status, sex) since there was high cutting mortality, both in control plants and in plants treated with IBA. At 120 DAS, all live cuttings still had the same leaves from the beginning of the rooting process. Thus, in this species, the presence of leaves is important for root induction, as cuttings without leaves or that had leaf abscission during the evaluated period did not survive.

Cuttings considered alive (with green leaves) in the control treatment and with IBA showed an average diameter at the base between 3.20 and 3.30 mm, respectively. Concerning dead cuttings, in the control treatment and in the treatment with IBA, these values averaged 3.20 and 3.26 mm, respectively. This way, the cuttings standardization was efficient and did not influence the results.

This work is pioneer in studying the adventitious rooting of *M. nigrum*, and demonstrated the possibility of propagation of this species through this technique. Although the percentage of rooting was not high, the ability to induce roots seems to vary with the genotype and, possibly, with the ontogenetic phase of the individual. Many studies with plants of the genus *Populus* (Salicaceae) have shown that the formation of adventitious roots is subject to a strong genotypic effect, and the ability to root varies significantly between different genotypes (Bannoud and Bellini, 2021). Similarly, this may have also occurred in plants of *Melanopsidium nigrum*.

The physiological and biochemical quality of matrix trees affects rooting capacity of cuttings. As it was impossible to determine the age of the matrix plants used, perhaps many matrices of *M. nigrum* were in a developmental stage with reduced adventitious rooting ability. The ontogenetic age of the matrix trees interferes with the formation of adventitious roots, as the cells that form the adventitious roots (mainly cambial cells or vascular parenchyma cells) lose competence for *de novo* regeneration of roots with the age and maturation of the tree (Díaz-Sala, 2014).

There was no effect of IBA on the induction of adventitious rooting of *M. nigrum*. In any case, auxin accumulation is crucial to induce the initial cells that will give rise to the primordia of adventitious roots (Gonin et al., 2019). The concentration (6000 mg.Kg^{-1}) used in this work was inefficient for activating new initial cells, or this concentration may have been high to the point of causing inhibition of induction. The estimated age and sex of the matrices should be considered in the future in the design of the experiments, as well as the comparison between the survival of individuals from the production of clonal seedlings concerning the survival of individuals transplanted manually to the RPPN Caruara.

The creation of protocols to obtain greater rooting of this species must take into account other concentrations of IBA, as well as the use of other compounds, which act on the content of antioxidant enzymes, such as hydrogen peroxide, or the control of rooting inhibitors. The use of pruning to stimulate more tender shoots and the management of solar irradiation control should be tested as future strategies for inducing adventitious rooting in this species.

The application of this technique in the management of the species in question brings a new perspective to the practices currently adopted in the installation of enterprises in areas with native vegetation and/or the occurrence of isolated individuals of species of threatened flora.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Bannoud, F., Bellini, C., 2021. Adventitious rooting in Populus species: update and perspectives. Front. Plant Sci. 12 https://doi.org/10.3389/fpls.2021.668837.

Bonga, J.M., 2016. Conifer clonal propagation in tree improvement programs. In: Park, Y.-S., Bonga, J.M., Moon, H.-K. (Eds.), Vegetative Propagation of Forest Trees. National Institute of Forest Science (NIFoS), Seoul, Korea, pp. 3–31.

Cabral, D. de C., Fiszon, J.T., 2004. Deforestation socio-spatial patterns and their implications to forest fragmentation: case study in Macacu River Basin, RJ. Sci. For. 66, 13–24.

Delprete, P.G., 2000. Melanopsidium Colla (Rubiaceae, Gardenieae): a monospecific Brazilian genus with a complex nomenclatural history. Brittonia 52, 325–336.

Díaz-Sala, C., 2014. Direct reprogramming of adult somatic cells toward adventitious root formation in forest tree species: the effect of the juvenile-adult transition. Front. Plant Sci. 5 https://doi.org/10.3389/fpls.2014.00310.

Gonin Bergougnoux, Nguyen, Gantet, Champion, 2019. What Makes Adventitious Roots? https://doi.org/10.3390/plants8070240.

Guedes, D., Barbosa, L.M., Martins, S.E., 2006. Composição florística e estrutura fitossociológica de dois fragmentos de floresta de restinga no Município de Bertioga, SP, Brasil. Acta Bot. Bras. 20 https://doi.org/10.1590/S0102-33062006000200006.

Hope, R.M., 2013. Rmisc: Ryan Miscellaneous. R Package Version 1.5.

Martinelli, G., Moraes, M.A., 2013. Livro vermelho da Flora do Brasil, first ed. Andrea Jakobsson: Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

R Core team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Thomazi, R.D., Rocha, R.T., Oliveira, M.V., Bruno, A.S., Silva, A.G., 2013. Um panorama da vegetação das restingas do Espírito Santo no contexto do litoral brasileiro. Natureza online 11 (1), 1–6.

Veloso, H.P., Rangel Filho, A.L.R., Lima, J.C.A., 1991. Classificação da vegetação brasileira adaptada a um Sistema Universal, CDDI-IBGE. IBGE, Rio de Janeiro.

Zappi, D., 2012. Melanopsidium nigrum in Melanopsidium (Rubiaceae) In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/2012/FB014116.

Zappi, D., Barbosa, M.R.V., Calió, M.F., Jardim, J.G., Pereira, M. do S., Souza, E.B. de, 2009. Rubiaceae. In: Zappi, D., Barbosa, M.R.V., Calió, M.F., Forzza, R.C., Stehmann, J.R., Salino, A. (Eds.), Plantas Da Floresta Atlântica, pp. 449–461. Rio de Janeiro.