- Short communication
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Rooting of the Endemic Brazilian Species *Campomanesia pubescens* using Biotechnological Techniques

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Campomanesia pubescens is a species from the Myrtaceae family, found in the Cerrado, Brazil. Popularly known as gabirobeira (Crispim et al. 2018), it is a fruit shrub tree, and its fruits are consumed *in natura* as well as manufacturing of pastries and liqueurs. It is widely used for reforestation of degraded areas. Locally it is also used for charcoal and firewood production. Furthermore, their leaves have medicinal properties, most commonly used in folk medicine to combat diarrhea, fever, scurvy and diseases of the urinary tract (Catelan et al. 2018).

Among the micropropagation stages, rooting is usually the step that shows more difficulties, especially in woody species (Singh and Agarwal 2016). Studies suggest that this difficulty is due to the recalcitrance of some metabolites when manipulated *in vitro* (Moyo et al. 2011). After the rooting step, plants usually need to go through the acclimatization stage which is the phase where the plants coming from *in vivo* conditions acquires all requirements to grow as a normal plant in the field. In this context, the aim of this study was to establish an *in vitro* and *ex vitro* rooting protocol for gabirobeira seedlings.

To establish a rooting and acclimatization protocol we used gabirobeira seedlings maintained *in vitro* in the Laboratory of Tissue Culture at the Federal University of Lavras, MG, Brazil. Several treatments were performed aimed to root *in vitro* gabirobeira seedlings, and are described in Table 1.

In all the above described rooting experiments, the culture media were supplemented with 0.9 M sucrose and 0.7% of agar. The pH was adjusted to 5.8 ± 0.1 before autoclaving at 121°C for 20 min. The explants were maintained in a growth chamber at 25 ± 2°C and photoperiod of 16h during six weeks, with evaluations every two weeks. The evaluated parameters were: the percentage of rooted shoots, leaf number and sprouting length.

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In vitro gabirobeira seedlings that did not show root formation in any of the treatment mentioned above, were used as explant source for *ex vitro* rooting and acclimatization using the commercial rooting powder Rootone®, which has a high concentration of IBA (3.000 ppm). The basal part of the shoots were treated with the powder and then placed in bottles containing vermiculite or Plantmax®.

Table 1. Treatments of pre rooting and rooting of all experiments evaluated.

Pre rooting		Rooting							
		4.4 µM IBA (6 Weeks)	IBA µM						Growth chamber (6 weeks)
2 Subcultures	4.4 µM BAP		0.0	1.5	3.0	4.5	6.0	7.5	9.0	
2 Subcultures	4.4 µM BAP	4.4 µM IAA (1 Week)	NAA µM							No auxin (6 weeks)
			0.0	1.0	5.0	10	20	100	_	
2 Subcultures	_	IBA + 1 µM Activated Charcoal (1 week)	IBA µM							No auxin (6 weeks)
			0.0	1.0	5.0	10	20	_	_	
2 Subcultures	_	IAA + 1 µM Activated Charcoal (1 week)	IAA µM							No auxin (6 weeks)
			0.0	1.0	5.0	10	20	_	_	

These plants were kept for 45 days in the containers wrapped with transparent plastic bag as pre-acclimatization in a growth chamber with 25 ± 2°C, photon irradiance at 67 µm $m^{-2}s^{-1}$ and 16 hours of photoperiod. After these 45 days, the plastic bags were removed and the plants were maintained for another 45 days in the same growth chamber at the same conditions. After these 90 days, were evaluated shoot length, number of leaves, number of roots, primary root length, fresh and dry weight. Were used ten replications per each treatment. The experimental design was completely randomized and the data were analyzed by ANOVA and were submitted to the Scott-Knott test at 0.05%, using the software Statsoft (Weiß 2007).

After 6 weeks in the growth chamber, we verified root formation only at the treatment with IBA. The treatments with 1.5, 4.5 and 6 µM of IBA demonstrated higher root average, with 28% 53% and 33% respectively (Fig. 1).

At the end of 90 days it was observed that 100% of gabirobeira seedlings transferred to *ex vitro* conditions survived the acclimatization regardless the used substrate and in 75% there were root formation (Fig. 2). For the other parameters (shoot length, leaves number, formed roots, main root length, fresh and dry), the different types of substrates tested were not statistically different from each other. Regarding the number of shoots and the length of the shoot, there was an increase by the end of the acclimatization

period, showing that the seedlings have adapted to the *ex vitro* environment. As with the previous parameters, the fresh mass and shoot dry mass did not differ statistically.

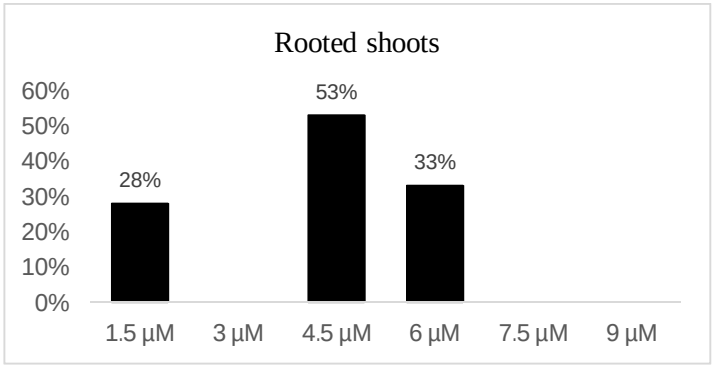

Fig. 1. Percentage of the number of gabirobeira rooted on MS medium with different IBA concentrations after 6 weeks.

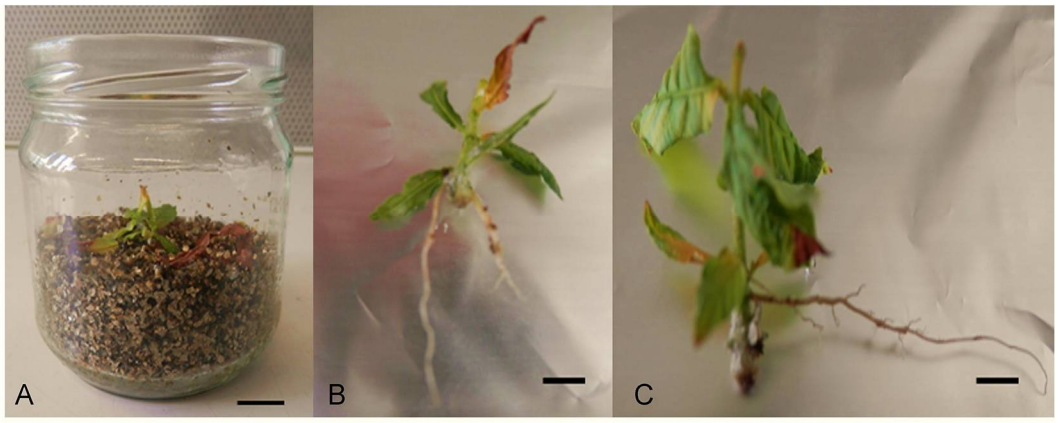

Fig. 2A. Gabiroba after rooting in *in vitro* condition, pré transferred to ex vitro. B-C. Gabiroba seedling after 90 days of rooting and ready to planting.

The growth regulator IBA has been the most common auxin used for *in vitro* rooting in different species being successful even with the eucalyptus species (Borges et al. 2012, Scutti and Zanette 2000). In a study with *C. xanthocarpa*, it was possible to obtain 53% of rooting from cuttings using WPM medium with IBA (Machado et al. 2020).

There are some studies indicating that the number of *in vitro* subcultures directly affects the rooting capacity of shoots (Hou et al. 2010). Although most studies report a rooting increase with rising subcultures (Guiyun and Sujuan 2007). The results obtained in *C. pubescens* show that the subcultures number, negatively influences on *in vitro* rooting for this species. Practical experience with micropropagation in several cerrado species has shown the negative influence of increasing the number of subcultures. This

finding can be explained by the fact that subcultures can increase the production of phenolic compounds, especially in woody species, which hinders the *in vitro* maintenance (George et al. 2008).

According to De Klerke (2002), the problems encountered during the rooting phase in micropropagation, consists one of the constraints to the plants transfer from *in vitro* to *ex vitro*. Thus, results such as those obtained in our work is of great interest because the union of acclimatization phase along with the rooting phase may optimize the time and cost for plantlets production.

Pedrotti and Voltolini (2001), investigating the acclimatization and *ex vitro* rooting in apple rootstock, obtained 64% rooting in the auxin absence, which is similar to the results found in this study. However, with the use of 1000 mg/l of IBA, was observed an increase in the rooting to 84%.

In our study, we were able to establish an *in vitro* (with IBA) and *ex vitro* rooting protocol for Gabirobeira. Gabirobeiras can be acclimatized and successfully rooted, with 100% survival and 75% rooting using Plantmax® or vermiculite substrate in a period of 90 days.

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