### **POSTER PRESENTATION**



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# Micropropagation of Pinus taeda L. via axillary buds

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#### Introduction

Pinus taeda stands for productivity and quality of its timber [1]. Researches using biotechnology are of great importance and have been applied to the improvement of its timber and plantation [2]. The main method of Pinus propagation is by seeds, once the minicuttings depends on the season of the year or depends of juvenile material [3-5]. Thus, researches on micropropagation of Pinus taeda are currently a priority in Brazil [6]. Micropropagation is the best method for mass production of superior genotypes and represents a strategy for tree improvement and capture of genetic gains [7]. Studies on Pinus taeda micropropagation by axillary bud proliferation are quite few. The purpose of this study was to develop a protocol for micropropagation of this species from juvenile material.

#### Materials and methods

For in vitro establishment two to four month old seedlings were used. Apical shoots and nodal segments of 3 cm length were inoculated in MS [8], DCR [9], WV3 [10] or WV5 [11] medium. For axillary shoots induction, the explants were inoculated in WV3, WV5 or DCR medium, with BAP (0, 0.12, 0.25 and 0.50  $\mu$ M). For the induction of roots, we tested the effect of double-layer medium, with semi-solid phase consisting of agar and water or GDm/2 [12] medium and the liquid phase containing water or GDm/2 medium. Both phases were supplemented with 2.69  $\mu M$  NAA and 0.44  $\mu M$  BAP for 9 days, followed by transfer to growth regulator-free GDm/2 medium. The rooted plants were planted in

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Plantmax<sup>®</sup> Forestry substrate and maintained in a greenhouse.

#### **Results and Discussion**

Nodal segments showed better responses during in vitro establishment, with up to 100% of explants forming axillary shoots and an average of 4.3 to 5.8 shoots per explant. The WV5 media proved better and presented the highest survival rate (86.0%) and highest elongation percentage (85.2%) (Figure 1).



medium, after nine weeks in the in vitro establishment. Bar: 1 cm.



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dies to optimize the rooting stage.

100 explants, in seven months of cultivation. The best rooting percentage (37.5%) was obtained in shoots treated with 2.69  $\mu M$  NAA and 0.44  $\mu M$  BAP for 9 days in culture medium composed of water and agar without liquid phase, followed by transfer to growth regulatorfree GDm/2 medium. The double-layer medium did not increase the rooting percentage. This result was higher than that found in *Pinus virginiana*, when the same combination of plant growth regulators was used [13]. The roots originated directly and indirectly from the stem with callus formation. After 90 days of acclimatization, the survival rate was 90% and an average of 4.6 roots per plant was obtained (Figure 2). This result was better than that obtained in other study with Pinus taeda that reported 38% of necrosis five weeks after transplantation [14]. Micropropagation of *Pinus taeda* from axillary buds and juvenile material is feasible, but requires further stu-

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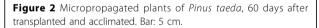
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The balance of salts in WV5 and WV3 culture media

favored an optimal development of in vitro cultures of

Pinus taeda due to its lower concentration of N in com-

parison with MS medium and to higher concentrations

of thiamine and inositol, which are growth promoters.

Elongated shoots were subdivided into segments, increas-

ing the multiplication rate to 3 segments per shoot. The

majority of BAP treatments did not promote better mul-

tiplication when compared to control. However, the

alternate use of 0.12  $\mu$ M BAP added to WV5 culture

medium during initial culture and a BAP-free medium

during the 1<sup>st</sup> subculture can increase the multiplication rate. The estimated production was 1024 shoots from



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