IN VITRO ESTABLISHMENT AND MULTIPLICATION OF THE HYBRID *EUCALYPTUS BENTHAMII* MAIDEN & CAMBAGE X *E. DUNNII* MAIDEN

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The establishment and multiplication are essential steps in the micropropagation of wood species. The aim of this work was to test concentrations of sodium hypochlorite ($NaOCl_2$) during disinfestations, and combination of BA and NAA in the multiplication rate of the spontaneous hybrid *Eucalyptus benthamiii* x *E. dunnii*. Young sprouts of ministumps of three clones (H12, H19 and H20) produced by cuttings were the source of explants. The ministumps were cultivated in semi-hydroponics system. Nodal segments with 2 axillary shoots (1.5 cm in length) and without leaf were used as explants. Four concentrations of $NaOCl_2$ (0.5, 1.0, 1.5, and 2.0%) and twelve combinations of BA and NAA (0, 1, 2 and 3 μmol L^{-1} of BA with 0, 0.1 and 0.2 μmol L^{-1} de NAA, only at clone H12) were tested. The explants were inoculated in MS and JADS mediums in the establishment and multiplication steps respectively, both mediums were supplemented with 250 mg L^{-1} of PVP40 and 30 g L^{-1} of sucrose, fixed in 6 g L^{-1} of agar at pH 5.8, and incubated at 25°C (±2°C), photoperiod of 16 hours in light with luminosity of 84μmol m^{-2} s^{-1}. Establishment disinfestations: the explants were immersed in ethanol solution 70% for 15s, rinsed with autoclaved water and then immersed in the treatments of $NaOCl_2$ during 10 min. Then, they were rinsed with autoclaved water for 3 times and inoculated in tubes (10 cm x 2 cm) with 10 mL of medium. Three typical responses of explants were evaluated weekly (contamination, oxidation and healthy) and after 21 days the length of the shoot buds were measured. All treatments were effective with no statistical difference. In general, the rate of bacteria contamination and oxidation were very low. The fungi contamination was about 41%. The healthy explants of clone H20 (66%) were bigger than clones H12 (45%) and H19 (46%). The biggest length of shoots (0.9 cm) was estimated in the concentration of 1.27% of $NaOCl_2$. Multiplication steps: the shoots of clone H12 were inoculated in tubes (10 cm x 10 cm) in 20 mL of medium. After 35 days of culture the number of shoots and the mass of the explants were evaluated. There was an interaction between the growth regulators used. The best combination was 2 μmol L^{-1} of BA with 0.2 μmol L^{-1} de NAA, which yielded about 6 shoots per explant with a mass of explant around of 50.91 mg. In summary, the contamination of explants used in this work was not a problem, and the multiplication of explant was viable with a rate of 6 shoots per explant in the best treatment.