

B-81 Some aspects of *in vitro* propagation of *Diospyros ebenum* (L)

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Ebony (*Diospyros ebenum* L.) is an endangered plant species for which no viable method of regeneration is available. In this study *in vitro* propagation of ebony through axillary bud cultures was investigated.

Immature axillary buds from the new flush were used as the explants. Two surface sterilization durations, (20 and 26 min), were tested. Axillary buds were established on half strength MS medium supplemented with 1 mg/l BAP with 0, 100, 200 and 300 $\mu\text{mol/l}$ and 25, 50 and 75 $\mu\text{mol/l}$ silver thiosulfate in 2 different experiments. Also axillary shoots were established in the MS medium with 2 mg/l BAP and 0.2% w/v charcoal. Multiplication of ebony was attempted on half strength MS medium with silver thiosulfate 25, 50 and 70 $\mu\text{mol/l}$ and 0, 2.5 and 5 mg/l BAP.

Bacterial contamination in cultures decreased from 46% to 16% with the increased duration of surface sterilization. Silver thiosulfate concentrations greater than 100 $\mu\text{mol/l}$ caused 71-43% of the initial survival of cultures. Leaf curling and necrosis followed by death were observed in silver thiosulfate free medium. The shoots established on 25 - 75 $\mu\text{mol/l}$ had low incidence of such symptoms and grew normal. Incorporation of charcoal reduced basal browning. Three BAP levels on multiplication did not show any shoot proliferation. However, 2.5 mg/l BAP in all 3 levels of silver thiosulfate showed 88% of culture survival in contrast to 50 and 12% in 1 and 5 mg/l BAP, respectively. Morphologically shoots on 50 $\mu\text{mol/l}$ silver thiosulfate were healthier than the other 2 levels.

Therefore, silver thiosulfate should be incorporated in half strength MS medium with 1 - 2.5 mg/l BAP at a rate of 50 $\mu\text{mol/l}$ in the establishment of ebony *in vitro* cultures.