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Micropropagation of a recalcitrant tree, *Salix alba* L.

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Objectives

This study was carried out to establish successful initiation and proliferation of culture and regeneration of plantlets from the culture. In addition, it was conducted to investigate the physiological factors that are related to recalcitrance of *Salix alba* L. in in vitro culture.

Materials and Methods

Plant material *Salix alba* L. clone 131-25

Steps for in vitro proliferation

Step	Culture	Problem	Experiment for overcoming
Culture initiation	Nodal culture	Dormancy	Bud-break
Maintenance of culture	Shoot proliferation Rooting	Necrosis	Rejuvenility
Regeneration from somatic tissues	Leaf, internodal segment, root segment and root-tip	Recalcitrance	PGRs, remove the volatile components etc.

Culture conditions 24±1°C, 16 h photoperiod, 35-40 mol m⁻² s⁻¹ photosynthetic photon flux

Results and Discussion

Propagules of *Salix alba* L. were produced by *in vitro* axillary shoot proliferation followed by rooting. Axillary bud-break in a single-node segment was strongly influenced by low levels of BA application but not by GA or chilling. Axillary buds were apparently in paradormancy phases induced by apical dominance. Shoots induced from nodal culture were placed on hormone-free medium for further growth. However shoot apex necrosis occurred after repeated subcultures. Lack of endogenous cytokinins appeared to be a responsible for rapid senescence or occurrence of tip necrosis. WPM containing 0.5 mg/L BA proved to be effective in overcoming shoot necrosis. When plantlets proliferated on BA were transferred on hormone-free WPM containing 0.1% activate charcoal, most of the explants successfully developed new shoots and roots within 4 weeks. Various tissues of well-developed plantlets were also tested for shoot regeneration. In six experiments comprising over 45 different treatment combinations of growth regulators and other factors, no shoots were regenerated except some calli and roots. The present study indicates that *Salix alba* is so recalcitrant that it might be needed some unconventional treatments to regenerate shoots from the culture in this species.

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