

Adventitious shoot regeneration in *Lotus glaber* Mill.

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In order to get a suitable protocol of regeneration for the genetic transformation of *Lotus glaber* Mill., several experiments from different explants were conducted. Roots, cotyledons and leaves from seedlings grown *in vitro* were cut into pieces and used as source of explants. Subsequently, they were cultured on 11 cc glass tubes containing 3 ml of Murashige and Skoog (1962) (plus sucrose 3%) semisolid medium (agar 0.65%), supplemented with different combinations of auxins (either naphthalenacetic acid or indoleacetic acid; NAA and IAA, respectively) and cytokinins (benciladenine, kinetin or thidiazuron; BA, KIN and TDZ, in that order). The cultures were incubated under $116 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF (photoperiod 14 h) and $27\pm 2^\circ\text{C}$.

After 45 days of culture, *de novo* shoot organogenesis was noticed from all explants tested. The type of explants markedly influenced organogenesis and the growth of the regenerated shoots. The regeneration frequencies were higher with leaf and cotyledons explants while the number of shoots formed per responsive explant was greater with leaf and roots.

The number of shoots produced per responsive leaf explant increased from 4 to 23, as the percentage of leaf explants producing shoots increased from 10 to more than 40%. NAA in combination with BA induced the highest regeneration rate ($40.1\pm 18.3\%$) bringing 19.7 ± 3.3 shoots per responsive leaf explant. Histological examination confirmed the direct process of organogenesis. The regenerated shoots from the best induction treatment were transferred to a fresh medium of similar chemical composition and without plant growth regulators for 30 days; in which, the *in vitro* rooting was stimulated. In all cases, the morphogenetic process was characterized by a direct pattern of root formation without callus proliferation. Plantlets with fully expanded leaves and well-developed roots were acclimatized in pots with transparent covers that were subsequently lifted to reduce humidity. The acclimatized plantlets were successfully established in soil. All plantlets were phenotypically normal.

In conclusion, our study provides a practical technique for efficient plantlets production of *Lotus glaber*. The procedure described here for the direct shoot organogenesis from various explants, and subsequently plantlets regeneration, facilitates the rapid propagation of this species. It will also be of use in cryoconservation and genetic breeding aimed at improving the abiotic stress tolerance.

References

MURASHIGE T. and SKOOG F.A. 1962. A revised medium for rapid growth and bioassays with tobacco culture. *Physiologia Plantarum*, **15**, 473-497.