Picloram-Induced Plantlet Regeneration of *Cypripedium calceolus* Through Root Tip Culture In Vitro

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Picloram has auxin action similar in effect to 2,4-D. It can serve as the auxin source in tissue culture with a number of species, but its primary value has been that it is effective at lower concentrations than 2,4-D and thus less likely to cause genetic changes.

Cypripedium calceolus is an endangered species and one that is difficult to propagate vegetatively via in vitro culture. Techniques were investigated to achieve a rapid propagation system for this species and we were successful in regenerating plantlets from root tips exposed to Picloram.

Seedlings of *C. calceolus* raised from immature seed were used. These had been removed from aseptic culture and kept in sealed vinyl bags in the dark at 5C for about 3 months. After surface sterilization with CaOCl for 20 min, root tips (ca 5 to 10 mm long) were cultured on half-strength MS medium supplemented with different concentrations of Picloram or 2,4-D (0,10, and 50 mg $liter^{-1}$) combined with BAP (1 or 10 mg $liter^{-1}$). All media were supplemented with 20 g $liter^{-1}$ sucrose, solidified with agar (7 g $liter^{-1}$) or Gellan gum (3 g $liter^{-1}$), with pH adjusted to 5.5 before autoclaving. All cultures were kept in the dark at 20C.

Initial callus proliferation was observed on the media with Picloram or 2,4-D combined with BAP, solidified with Gellan gum after 4 weeks of culture. Almost all calli on the medium with 2,4-D turned brown and died after 8 weeks of culture. Calli survived on the media supplemented with Picloram and BAP. Where BAP was kept constant and Picloram increased, an increase in the rate of callus formation was noted. On the surface of growing calli, shoot proliferation was observed after 16 weeks.

After 22 weeks of culture, root differentiation was observed on the base of some shoots. These plantlets and shoots were separated from root-tip explants and transferred onto half-strength MS medium (plant growth regulator free) with Gellan gum (3 g $liter^{-1}$). Roots differentiated and plantlets were established after a further 4 weeks of culture.

These results demonstrate a novel method with potential for vegetative propagation of *C. calceolus* via in vitro culture.