

PROTOPLAST CULTURE OF SUNFLOWER (*Helianthus annuus* L.).

Lénée, P., Chupeau, Y. Laboratoire de Biologie cellulaire, I.N.R.A.
F-78000 Versailles (France).

We have initiated studies on the isolation and culture of protoplasts in the genus *Helianthus* which belongs to the economically important Compositae family. Our first results with different *Helianthus* tissues are briefly described here. Except for cotyledons (2×10^5 protoplasts/g fresh weight) and stems (1×10^5 protoplasts/g fresh weight), yields of protoplasts were high: 8×10^6 protoplasts/g fresh weight from leaves and 5×10^6 protoplasts/g fresh weight from hypocotyls. No division were observed in protoplasts from mesophyll, stems and cotyledons cultured on several media in which nitrogen source and concentration, as well as NAA, BA and mannitol concentrations were varied. Conversely, protoplasts derived from hypocotyl began to divide after 4 days of culture in all the media tested. The percentage of plated protoplasts developed to multicellular colonies after 10 days varied between 20 and 60% depending on the experiment. However after that time all the colonies obtained on a medium containing ammonium nitrate (5 mM) and nitrate (5 mM) died by the 2nd week of culture. Approximately 1% of the protoplasts plated on a medium containing glutamine (7,5 mM) as sole source of nitrogen gave rise to micro-calli transferable on to solid media for regeneration attempts. It is possible to enhance the formation of viable calli from 1% to 10% the initially plated protoplasts by early subculture on a glutamine medium. Assay of nitrate reductase activity of cells derived from protoplasts on ammonium nitrate medium shows a decrease of the activity during the first 10 days of culture. In our work neither early protoplast development nor callus formation were problematical for the various lines and hybrids tested. The striking observation in this study in the apparent toxicity of nitrate for *Helianthus annuus* cells. This feature is a limiting step in regenerating plants and is thus a barrier for use of protoplasts as a new plant breeding tool in the genus *Helianthus*. These preliminary results emphasize the necessity for precise studies on nitrogen assimilation by protoplasts and cells derived from sunflower hypocotyls.

PROTOPLAST CULTURE OF SUNFLOWER (*Helianthus annuus* L.)

Lénée, P., Chupeau, Y.

Lénée Philippe
Laboratoire de Biologie cellulaire - INRA -
78000 Versailles (France).

Génétique et Amélioration des espèces.