PROTOCOLS & RECIPES
Protoplast Preparation

Protocol for the preparation of regeneration competent protoplasts

Method for the isolation of Brassica napus protoplasts from mesophyll material derived from one single individual plant (greenhouse grown plants or in vitro cultured shoots) or from young in vitro grown hypocotyls when differend individuals are acceptable.

When using leaf material a dark treatment (24 h to 48 h) is recommended for achieving an optimal mesophyll protoplast isolation. To obtain sterile hypocotyls, seeds are surface sterilized with 7.5 % calcium hypochlorite for 1 h with continuous shaking, treated with 70 % ethanol for 1 minute and then washed twice in sterile distilled water.

The seeds are germinated on MS-medium (Vamling et Glimelius, 1990). Hypocotyl and mesophyll materials are treated identically for protoplast isolation; in case of modifications for the mesophyll treatment the values are given in brackets.

(1) 4 to 5 days (mesophyll 30 days) old Hypocotyls are cut into 0.5 to 1 mm (1 to 2 mm) segments and incubated for 1 h in plasmolyse buffer (Vamling et Glimelius, 1990).

(2) After plasmolysing the material is treated with 1 % Cellulase and 0.1 % Macerozym in the medium K3 according to Nagy et Maliga, (1976), modified with the addition of 0.4 M sucrose. Hypocotyl material is incubated 16 to 18 h at 20 - 22 ℃ in the dark on a rocking tray with low speed. The mesophyll material is incubated under the same conditions, but without shaking and the time for enzyme treatment can be shortened to 4 - 5 h.

(3) The enzyme treated material is filtered through a nylon mesh (50 µm pore size) to separate undegraded material. To the protoplasts containing enzyme solution an equal volume of the salt solution CPW16 (Banks et Evans, 1976) is added.

(4) The mixed solutions are centrifuged in a swing-out rotor at 100 x g for 7 min.

(5) Protoplasts with intact cell membranes float on the surface and can be removed with a Pasteur pipette.

(6) The protoplast suspension is diluted about 10 times in the salt Solution W5 (Menczel et al. 1981).

(7) Washing of the protoplasts in the salt Solution W5 is repeated once by flotation and replacing the medium.

(8) The protoplasts are finally pelleted by centrifugation in culture medium KM8p, modified (Glimelius et al. 1986) and cultured at a density of 2.5 - 5.0 x 10^4 /ml in culture medium.

References:

1) Banks M.S. **and** Evans P.K. (1976) Plant Sci. Lett. **7,** 409-416
2) Glimelius K. et al. (1986) Plant Sci. **45,** 133 - 144
3) Menczel L. et al. (1981) Theor. Appl. Genet. **59,** 191 - 195
4) Vamling K. et Glimelius K. (1990)
   Legumes and Oilseed Crops I in Biotechnology in Agriculture and Forestry **10,** Springer Verlag

SERVA Electrophoresis GmbH ● Carl-Benz-Str.7 ● D-69115 Heidelberg
Telefon: +49 6221 13840-0 ● E-Mail: info@serva.de ● Internet: www.serva.de