Plant Cell Reports

Plantlets from Mesophyll Protoplasts of *Solanum xanthocarpum*

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Abstract

Young leaves of Solanum xanthocarpum from axenic shoot cultures released viable protoplasts when treated with appropriate enzymes. The protoplasts on culture in modified Murashige and Skoog (1962) medium supplemented with 2,4-dichlorophenoxy-acetic acid (0.5 mg/l), naphthaleneacetic acid (1 mg/l), kinetin (1 mg/l) and organic nutrients of KM (Kao and Michayluk 1975) regenerated to form callus tissue as a result of repeated divisions. Protoplast-derived calli differentiated into shoots on MS medium enriched with kinetin (0.5 mg/l) and rooting could be initiated by transferring the shoot-buds to basal medium.

Introduction

To genus Solanum belong many vegetable crops and alkaloid producing plants. This group therefore is a suitable system for genetic modification of plants employing novel techniques such as somatic cell hybridization. The observations reported in this communication are preliminary results in this direction on regeneration of plantlets from isolated protoplasts of S. xanthocarpum, a weed common in India but known to be of much medicinal importance.

Material and Methods

Seeds of Solanum xanthocarpum Schrad & Wendl. were germinated on MS (Murashige and Skoog 1962) basal medium, without hormones, under sterile conditions. For initiating shoot cultures, 10-day-old shoot tips were transferred to Murashige and Skoog (1962) medium supplemented with kinetin (1 mg/l) and indoleacetic acid (0.5 mg/l). Young buds (4-8), proliferating from shoot-tips, were subcultured in the same medium but supplemented with 0.2 mg/l kinetin for normal growth and expansion of the leaves. Cultures were illuminated at 3000 lux (16/8 h light and dark cycles) at 25°C.

Protoplasts were prepared by enzymatic digestion of one-month-old fully expanded leaves employing 1.5% cellulase (Onozuka R-10), 0.5% macerozyme (R-10) and 5 mM $CaCl_2$ in a solution containing mannitol (0.25M) and sorbitol (0.25M). The incubation was carried out in dark for 6-8 h at 25°C. The protoplast preparation was freed of debris by flotation on 20% sucrose solution, containing minerals of the culture medium, as described earlier (Gill et al. 1978). The protoplasts were then removed carefully by a Pasteur pipette and washed twice with the culture medium, which comprised minerals of MS medium but with a reduced level of NH_4NO_3 (270 mg/l), 2,4-dichlorophenoxyacetic acid (0.5 mg/l), naphthaleneacetic acid (1 mg/l), kinetin (1 mg/l) and organic nutrients of KM medium (Kao and Michayluk 1975). A mixture of sorbitol (0.25 M) and mannitol (0.25 M) served as osmoticum and 2% sucrose was included as the carbon source. Density of the suspension was adjusted to 2-4 x 10^4 protoplasts per ml. Drops of this suspension were cultured in sterile Petri dishes (35 x 10 mm) in a manner as reported for pepper protoplasts (Saxena et al. 1981b) and cultures were incubated in dark (Gill et al. 1981) for two weeks and later illuminated at 3000 lux (16/8 h light and dark cycles). Four-week-old cell colonies, developed from protoplasts, were transferred to MS medium (without mannitol or sorbitol but with 5% sucrose) and supplemented with naphthaleneacetic acid and kinetin at 1 mg/l each, for callus formation. The differentiation of shoot-buds from the calli was achieved on a differentiation medium (MS with 0.5 mg/l kinetin and 3% sucrose) and the shoots developed roots on the basal medium.

Results and Discussion

In Solanum xanthocarpum it was easy to establish rapidly growing axenic shoot cultures. En masse differentiation of shoot-buds was possible from various parts of the plants with little callusing. The leaves from axenic shoot cultures on enzymatic dissolution produced about 1-4 x 10^6 protoplasts (Fig. 1A) per gram of tissue. A dark pretreatment

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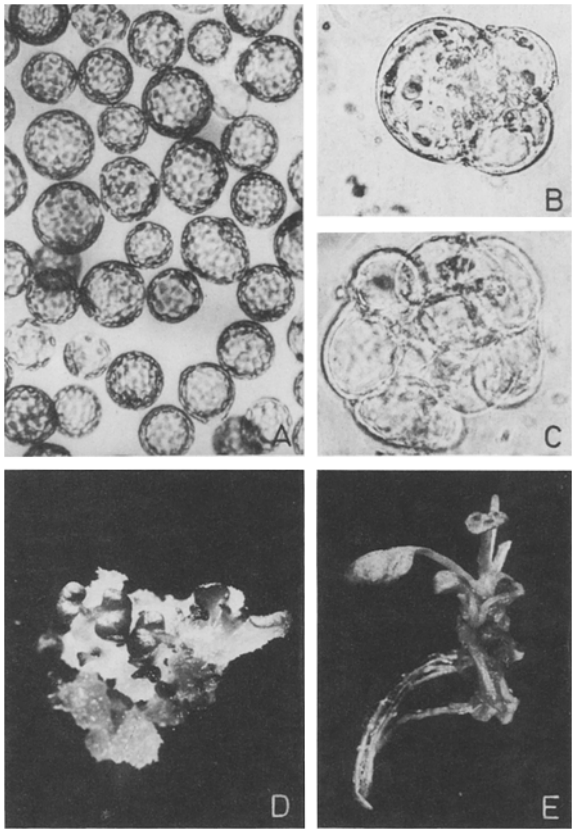

Fig. 1. Regeneration of plantlet from mesophyll protoplasts of Solanum xanthocarpum. A freshly isolated protoplasts. B initiation of cell divisions. C multicellular colony. D differentiation of shoot buds. E the regenerated plantlet.

of 7 days to shoot cultures was, however, necessary for the production of viable protoplasts. Yields of protoplasts from seed grown plants were poor and they divided occasionally to produce 2-4 cells only which did not grow further. Contrarily, as many as 20% of the protoplasts -- harvested from shoot cultures -- underwent first division (Fig. 1B) within 72 h and formed small colonies (Fig. 1C) in a week which developed into small calli in a month. On transfer to MS medium enriched with 1 mg/l naphthaleneacetic acid, 1 mg/l kinetin, and 5% sucrose (in place of mannitol and sorbitol) under illumination of 3000 lux light, the calli turned yellowish green. Appearance of shoot-bud primordia in 20-30% cultures on the differentiation medium (MS with kinetin 0.5 mg/l and 3% sucrose) was marked by the formation of dark green areas from which differentiated the shoot-buds (Fig. 1D) in about two weeks. When implanted on MS basal medium all the shoots developed roots (Fig. 1E). Thus, like other species of Solanum namely, S. tuberosum (Binding et al. 1978), S. dulcamara (Binding and Nehls 1977), S. nigrum (Nehls 1978) and S. melongena (Saxena et al. 1981a), the use of axenic cultures in Solanum xanthocarpum also is highly beneficial for successful regeneration of plants from isolated protoplasts.

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