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# Isolation and Culture of Protoplasts of *Capsicum annuum* L. and Their Regeneration into Plants Flowering *in vitro*

*Brief Report*

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## Summary

Axenic shoot cultures of *Capsicum annuum* cv. *California Wonder* were used as the source for isolation of protoplasts from mesophyll cells. Protoplasts underwent sustained mitotic activity and proliferated to form callus masses on NT or DPD medium enriched with 2,4-D, NAA and BAP each at 1 mg/l level. The callus could be differentiated into whole plants on the differentiation media and plants flowered *in vitro* under long day conditions.

*Keywords: Capsicum annuum;* Protoplast regeneration.

## 1. Introduction

Protoplast technology is now reckoned as a very important approach for crop improvement through mutation, somatic hybridization or genetic modification by uptake of foreign genome. Obviously, a basic requirement for achieving this goal is the successful regeneration of plants from isolated protoplasts. Following the report of plantlet formation from protoplast derived cells of *Nicotiana tabacum* (NAGATA and TAKEBE 1971), protoplasts of many species have been induced to develop into intact plants (for a comprehensive list see VASIL and VASIL 1980). Recently, however, attempts are increasingly being directed towards cultivation of protoplasts of the economically important plants. In this communication we report our preliminary observations on regeneration of plants from mesophyll protoplasts of red pepper, a plant of much economic importance.

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## 2. Material and Methods

The seeds of *Capsicum annuum* L. cv. *California Wonder* were obtained from the Indian Agricultural Research Institute, New Delhi and sown aseptically on MURASHIGE and SKOOG (1962) basal medium. Then the excised shoot tips were inoculated on MS medium supplemented with 3 mg/l kinetin, 0.5 mg/l IAA and 7% coconut water (obtained from green fruits) in 100 ml Erlenmeyer flasks (SAXENA *et al.* 1981). Essentially this procedure was designed to produce better foliage.

The preparation of protoplasts was accomplished by enzymatic dissociation of cells of 15-day-old leaves. For this, about 400 mg of the leaf tissue—cut into smaller segments each approximately 2 mm square—was incubated per 20 ml of incubation mixture which comprised MS mineral salts (diluted 10-fold), 2% cellulase (Onozuka R-10), 0.4% macerozyme (R-10) and 0.5 M mannitol for 8–10 hours. The protoplasts were cleaned by repeated centrifugation at 55 × g employing a culture medium which contained mineral salts of DPD (DURAND *et al.* 1973) or NT (NAGATA and TAKEBE 1971) supplemented with 2,4-D, NAA, BAP (each at 1 mg/l), 2% sucrose and 0.5 M mannitol.

The density of the suspension of protoplasts was adjusted to $5 \times 10^4$ per ml and small drops of 0.1 to 0.2 ml were sandwitched between two layers of the 0.7% agar culture medium in sterile plastic petri dishes (9 cm diameter). The cultures were incubated at 25 ± 1.5 °C in the dark for 15 days. For differentiation the cultures were exposed again to light. For this purpose intensities between 3,000 and 5,000 lux seemed equally satisfactory—at least no marked effect could be noted.

## 3. Results and Discussion

Axenic shoot cultures were employed as the source for isolation of protoplasts. Attempts to isolate viable protoplasts revealed that the growth conditions of donor plants had marked influence on the yield of protoplasts and their subsequent regeneration. Only those shoot cultures exposed to an illumination of around 3,000 lux were appropriate—lower or higher intensities, *e.g.*, 2,000 or 5,000 lux reduced the yields.

Cell wall regeneration around the protoplasts, as detected by staining with Calcofluor-White ST (NAGATA and TAKEBE 1971), occurred within 24 hours and the divisions commenced in 4–7 days (Figs. 1 *A*, *B*, and *C*). About 2 to 5% protoplasts underwent sustained proliferation. The tiny cell clusters (Fig. 1 *D*) when transferred to low osmotic medium (0.25 M mannitol) and illuminated at 3,000 lux, underwent rapid proliferation and developed callus masses in one and a half month. For attainment of shoot morphogenesis (Fig. 1 *E*) calli were transferred on MS medium enriched with IAA (4 mg/l), kinetin (2.56 mg/l) and 3% sucrose (GILL *et al.* 1978). The shoots formed roots on a medium which had IAA (1 mg/l) and kinetin (0.04 mg/l). The plants, derived from protoplasts, when five months old (since the day of isolation), flowered (Fig. 1 *E*) under the long-day (16 : 8 hours light and dark cycle) condition of the culture room.

Axenic shoot cultures are now being increasingly recommended because they ensure relatively greater physiological uniformity of the source tissue and therefore produce viable protoplasts which show a high degree of mitotic

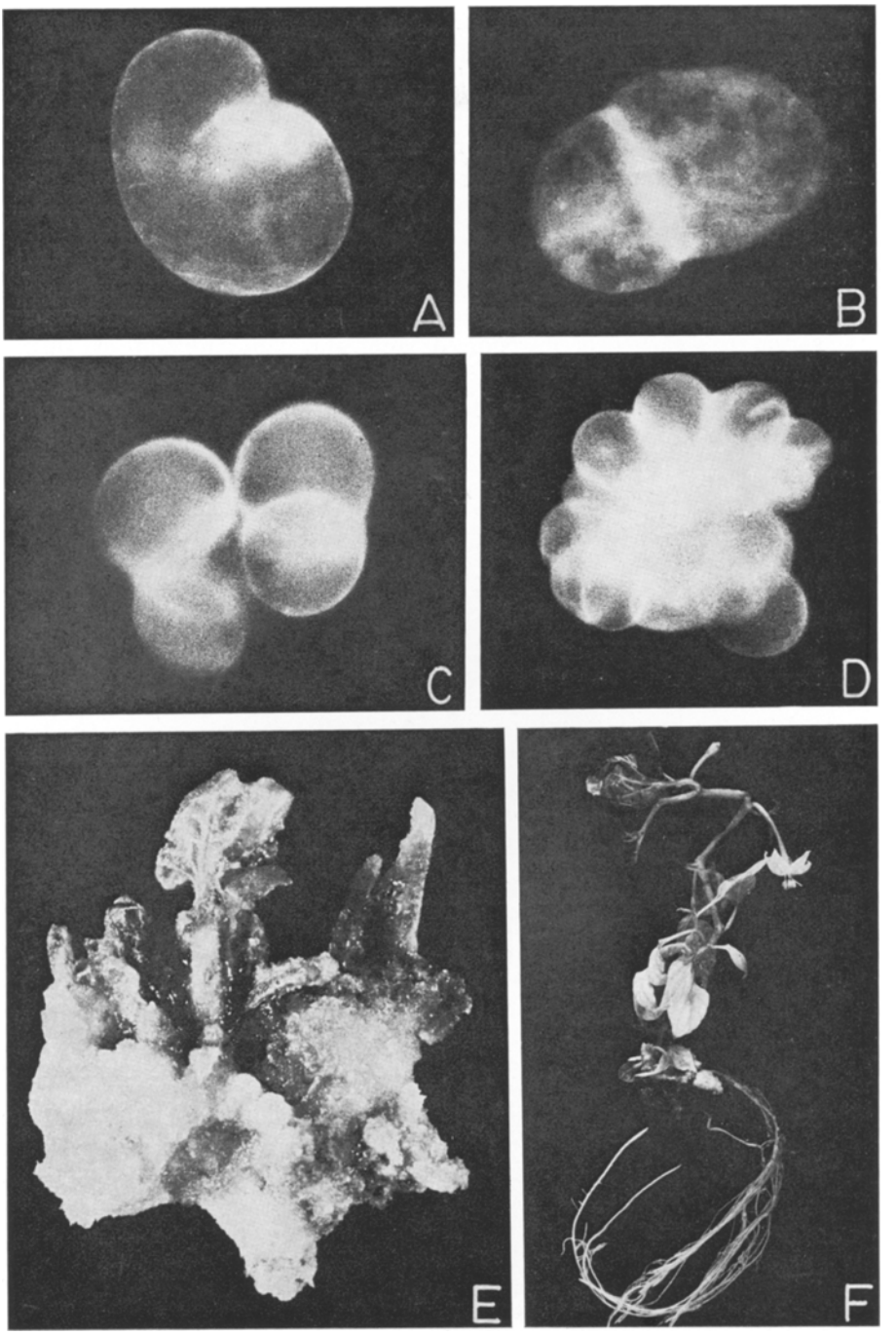


Fig. 1. Regeneration of protoplasts of *Capsicum annuum* to plants. *A* Fluorescent micrograph showing regeneration of cell wall and initiation of cell division after 3 days in DPD medium supplemented with 2,4-D, NAA and BAP (1 mg/l each). *B* A bicellular regenerant. *C, D* Multicellular colonies after two weeks. *E* Differentiation of shoot buds on MS medium enriched with kinetin (2.56 mg/l), IAA (4 mg/l) and 3% sucrose. *F* A plantlet with well-developed roots, shoot and flowers

activity (BINDING 1975, LÖRZ *et al.* 1979, SAXENA *et al.* 1981). A strong influence of the conditions of illumination of the donor plants on the yield of protoplasts and their subsequent regeneration has been emphasized also in several systems investigated earlier like *Petunia hybrida* (BINDING 1974), *Nicotiana tabacum* (BINDING 1975), *N. sylvestris* (DURAND 1979), *Hyoscyamus albus* and *H. muticus* (LÖRZ *et al.* 1979) *Solanum tuberosum* (SHEPARD and TOTTEN 1977), and *Solanum melongena* (SAXENA *et al.* 1981). As for *in vitro* flowering of protoplast-derived plants of *C. annuum*, a similar observation has earlier been reported from this laboratory in *N. rustica* (GILL *et al.* 1979).

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## References

BINDING, H., 1974: Regeneration von haploiden und diploiden Pflanzen aus Protoplasten von *Petunia hybrida* L. Z. Pflanzenphysiol. **74**, 327—356.

— 1975: Reproducibly high plating efficiency of isolated mesophyll protoplasts from shoot cultures of tobacco. Physiol. Plant. **35**, 225—227.

DURAND, J., 1979: High and reproducible plating efficiencies of protoplasts isolated from *in vitro* grown haploid *Nicotiana sylvestris*. Z. Pflanzenphysiol. **93**, 283—295.

— POTRYKUS, I., DONN, G., 1973: Plantes issues de protoplastes de *Pétunia*. Z. Pflanzenphysiol. **69**, 26—34.

GILL, R., RASHID, A., MAHESHWARI, S. C., 1978: Regeneration of plants from mesophyll protoplasts of *Nicotiana plumbaginifolia* Viv. Protoplasma **96**, 375—379.

— — — 1979: Isolation of mesophyll protoplasts of *Nicotiana rustica* and their regeneration into plants flowering *in vitro*. Physiol. Plant. **47**, 7—10.

LÖRZ, H., WERNICKE, W., POTRYKUS, I., 1979: Culture and plant regeneration of *Hyoscyamus* protoplasts. Planta Medica **36**, 21—29.

MURASHIGE, T., SKOOG, F., 1962: A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. **15**, 473—497.

NAGATA, T., TAKEBE, I., 1971: Plating of isolated tobacco mesophyll protoplasts on agar medium. Planta (Berl.) **99**, 12—20.

SAXENA, P. K., GILL, R., RASHID, A., MAHESHWARI, S. C., 1981: Plantlet formation from isolated protoplasts of *Solanum melongena* L. Protoplasma **106**, 355—359.

SHEPARD, J. F., TOTTEN, R. E., 1977: Mesophyll cell protoplasts of potato—isolation, proliferation, and plant regeneration. Pl. Physiol. **60**, 313—316.

VASIL, I. K., VASIL, V., 1980: Isolation and culture of protoplasts. Int. Rev. Cytol. (Suppl.) **11 B**, 1—18.