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PROTOPLASMA

Plantlet Formation from Isolated Protoplasts of *Solanum melongena* L.

Brief Report

P. K. SAXENA *, R. GILL, A. RASHID, and S. C. MAHESHWARI

Department of Botany, University of Delhi, Delhi

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Summary

Mesophyll protoplasts were isolated from axenic shoot cultures of *Solanum melongena* by the one-step enzymatic method. Of the different media employed for the culture of protoplasts, a medium modified from KAO and MICHAYLUK (1975) supported sustained mitotic cycles most effectively. Organogenesis from protoplast-derived callus was achieved on transfer to MURASHIGE and SKOOG's (1962) medium supplemented with an appropriate auxin and a cytokinin.

Keywords: Solanum melongena; Isolated protoplasts; Regeneration.

1. Introduction

The totipotency of plant protoplasts has provided a viable and important approach to the study of plant morphogenesis and crop improvement. In recent years, a great deal of work has been initiated on these aspects and the protoplasts of many plants—belonging to different families—have been induced to divide, proliferate and redifferentiate into whole plants. Currently, the emphasis is upon the regeneration of protoplasts of economically important plants.

The genus *Solanum* is of wide economic importance and has been investigated in detail for the development of plants from isolated protoplasts, *e.g.*, *Solanum tuberosum* (SHEPARD and TOTTEN 1977, BINDING *et al* 1978), *Solanum dulcamara* (BINDING and NEHLS 1977), and *Solanum nigrum* (NEHLS 1978). The present note concerns preliminary studies on plantlet formation from the protoplasts of an important vegetable crop plant, *Solanum melongena*.

* Correspondence and Reprints: Department of Botany, University of Delhi, Dehli-110007, India.

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2. Material and Methods

The seeds of *Solanum melongena* were obtained from IARI, New Delhi, and were sown aseptically on Murashige and Skoog's medium supplemented with kinetin (2 mg/l). Axenic shoot cultures were established as per the method of BINDING (1974). Shoot cultures were, however, raised in 100 ml Erlenmeyer flasks which were illuminated at 2,500–3,000 lux intensity by fluorescent lamps.

For the preparation of protoplasts, 2-week-old leaves were sliced into strips and incubated in an isolation mixture which comprised 1.5% cellulase (Onozuka R-10), 0.5% macerozyme (R-10), and 5 mM $CaCl_2$ in a 0.5 M mannitol solution. Incubation was carried out in the dark at 23 °C for 6–8 hours. The protoplasts were then separated from undigested material by sieving (GILL *et al.* 1978) and the resulting solution was centrifuged at 600 rpm for 3 minutes. The supernatant was discarded and the protoplast pellet was washed once with mannitol and twice with the culture medium (Table 1) having 0.5 M mannitol. About 0.3 ml of the suspension, at a density of 2–5 $\times$ 10^4 protoplasts per ml, was cultured in 5 ml glass vials.

The regenerants, when 15-day-old, were gently mixed with agar-gelled medium of the same composition and plated in glass petriplates (5 $\times$ 10 mm). After a month tiny calluses were transferred to differentiation medium. The cultures were kept in dark (23 °C) for the first 2 weeks and thereafter illuminated at 3,000 lux (25 $\pm$ 1.5 °C).

3. Results and Discussion

Studies on the isolation of protoplasts from *Solanum melongena* leaves indicated that the environment and nutritional milieu of the donor plant were critical factors for the recovery of viable protoplasts. The yield from

Table 1. *Composition of Protoplast Culture Medium*

Ingredients	Amount (mg/l)
a) Macronutrients	
NH_4NO_3	1,280
KNO_3	270
$CaCl_2 \cdot 2\,H_2O$	770
$MgSO_4 \cdot 7\,H_2O$	370
KH_2PO_4	180
b) Micronutrients	as per Heller's solution (1953)
$FeSO_4 \cdot 7\,H_2O$	2.78
Na_2EDTA	3.72
c) Hormones	
2,4-D	0.5
BAP	1.0
NAA	1.0
d) Sugars	as per KAO and MICHAYLUK (1975)
e) Vitamins	
f) Organic acids	
g) Casamino acids	
h) Coconut water	20 ml

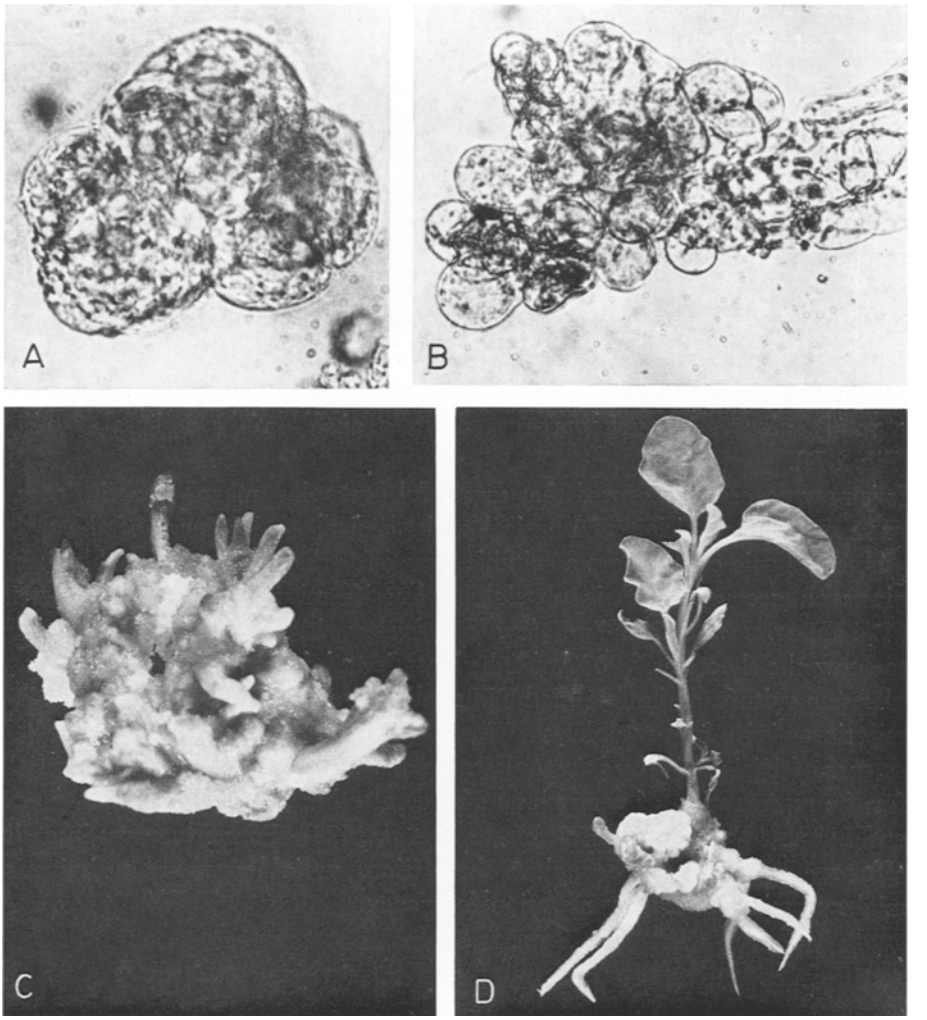

Fig. 1. Plantlet formation from isolated protoplasts of *Solanum melongena*: *A* one-week-old protoplast-derived regenerant, showing a few cells, in modified KM medium. *B* Callus mass developed on the same medium solidified with 0.6% agar. *C* Differentiation of callus on MS medium supplemented with kinetin (3 mg/l) and indoleacetic acid (0.5 mg/l). *D* A four-month-old protoplast-derived-plant growing on MS Basal medium

mesophyll tissue obtained from *in vitro* grown shoots, was better than from seed grown plants. Nevertheless, illumination of donor plants, *i.e.*, shoot cultures at 2,500–3,000 lux and addition of calcium chloride to the isolation mixture were required for a consistently good yield of protoplasts—2–5 $\times$ 10^6/g tissue.

Several media, routinely employed for protoplast culture, were screened in order to select a suitable one for *S. melongena*. Of these, NT, *i.e.*, the formula-

tion used by Nagata and Takebe (1971), DPD (Durand, Potrykus, and Donn 1973) and KM (Kao and Michayluk 1975) supported divisional activity of the protoplasts whereas others, *i.e.*, B_5 (Gamborg *et al.* 1968), ON (Ohyama and Nitsch 1972), and T_0 (Bourgin *et al.* 1979) did not. The regeneration frequency, however, was disappointingly low in NT and DPD media and only occasionally the regenerants proliferated to form callus masses. However, a modification of the KM medium (Tab. 1) permitted sustained mitotic cycles. About 20–30% of the protoplasts underwent division and callus formation (Figs. 1 *A* and *B*). Bicellular regenerants could be seen in 4–7 days and these formed callus in one and a half month on transfer to agar-gelled medium. The differentiation of shoots (Fig. 1 C) was achieved on MS medium supplemented with kinetin (3 mg/l) and IAA (0.5 mg/l) or BAP (1 mg/l) alone. The shoots rooted on transfer to MS basal medium (Fig. 1 D).

It is now generally accepted that successful protoplast morphogenesis depends upon extreme precision of physical and chemical environment of the donor plants. Axenic cultures are a suitable source for the isolation of viable protoplasts on account of their physiological uniformity and sterility (Binding 1974, Lörz *et al.* 1979). In *Solanum melongena* it was found to be necessary to grow the shoots at 3,000 lux light intensity to check the bursting of protoplasts on culture, and a modified KM medium was found suitable for sustained mitotic activity of protoplasts. KM medium was originally devised for the culture of *Vicia hajastana* protoplasts but has also been found to be beneficial for the regeneration of protoplasts of several other *Solanum* species (Binding and Nehls 1977, Nehls 1978, Binding *et al.* 1978). Presently, more intensive efforts are underway to explore the various variables whose optimization might permit maximal regeneration frequency.

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