Short Communication Plant Tissue Culture Letters, 4(1), 35-37 (1987)

Isolation and Culture of Protoplasts from *Luffa cylindrica* Suspension Cultures

Shogo MATSUMOTO,*,** Mitsuaki ITO,** Hiroaki KONISHI,** and Itaru TAKEBE*

* *Department of Biology, Faculty of Science, Nagoya University, Chikusa, Nagoya, 464 Japan*
** *Biochemical Research Institute, Nippon Menard Cosmetic Co.,Ltd., Ogaki, Gifu-ken, 503 Japan*

(Received October 4, 1986)
(Accepted November 20, 1986)

The family Cucurbitaceae contains a number of important vegetable and fruit crops, and some species including *Luffa cylindrica* have been used as a source of cosmetic materials. Although culture of protoplasts from cucumber and melon have been reported,[1-3] there is no report on the isolation and culture of *Luffa cylindrica* protoplasts. In this paper, we describe a procedure for successful isolation and culture of protoplasts from *Luffa cylindrica* suspension culture.

Seeds of *Luffa cylindrica* cv. *Futo* were surface sterilized in 2.5% (w/v) sodium hypochlorite solution for 10 min and washed twice in sterile distilled water. They were germinated on MS agar medium, and calli were induced by placing cotyledon segments of 2-3 week old axenic seedlings on MS agar medium supplemented with 1.0 mg/*l* 2,4-D and 0.5 mg/*l* kinetin. After 2-3 months of culture, tiny pieces of friable calli were transferred into liquid MS medium of the same composition to initiate suspension cultures. After repeated subcultures at 28℃ on a reciprocal shaker (90 strokes/min), they were maintained by regularly transfering 10 ml culture (about 3.0 g fr wt of cells) to 80 ml of fresh medium in a 300 ml Erlenmyer flask at 10 day intervals. The duration of lag period (3 days) and the growth rate have remained unchanged for more than a year.

For protoplast isolation cells were collected by centrifugation at $100\times g$ for 2 min, and washed once with 0.4 M mannitol. To the cells of 1 g fresh weight was added 2.5 ml of filter-sterilized enzyme solution containing 0.05% Pectolyase Y-23 (Seishin Pharmaceutical Co. Ltd., Tokyo, Japan), 0.5% Macerozyme R-10, 1.0% Cellulase Onozuka RS (Yakult Honsha Co. Ltd., Tokyo, Japan) and 0.4 M mannitol, pH 5.7. After 2 hr of incubation at 25℃ with occasional pipetting, undigested cell aggregates were removed by filtration through a 50 μ nylon sieve, and protoplasts were collected by centrifugation at $100\times g$ for 5 min, followed by washing twice with 0.4 M mannitol. The number of protoplasts was counted in a haemocytometer.

The growth phase of cultures strongly influenced the yield and viability of protoplasts, as has been reported in other species.[5,6] The highest yield of protoplasts (2×10^6 cells/g fr wt) was obtained at day 6 with viability of approximately 90% (**Fig. 1**).

The washed protoplasts were resuspended in liquid culture media at a cell density of 1×10^5/ml, and cultured in 2.4 ml portions in 60×15 mm plastic dishes (Falcon 1007) at 28℃ in diffuse light.

No cell division was observed when protoplasts were cultured in MS medium supplemented with 1.0 mg/*l* 2,4-D, 0.5 mg/*l* kinetin and 0.4 M mannitol. Maeda et al.[6] and Nakagawa et al.[7] reported that the osmolarity optimal for protoplast culture was lower than that for protoplast isolation. In accordance with this finding *Luffa cylindrica* protoplasts divided when mannitol concentration in the culture medium was reduced to 0.25 M. Since only 1-5% of total protoplasts divided in the

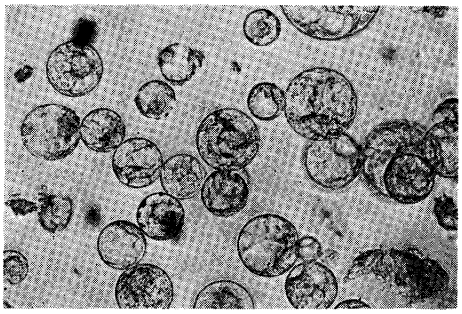

Fig. 1. Protoplasts from *Luffa cylindrica* suspension cultures.

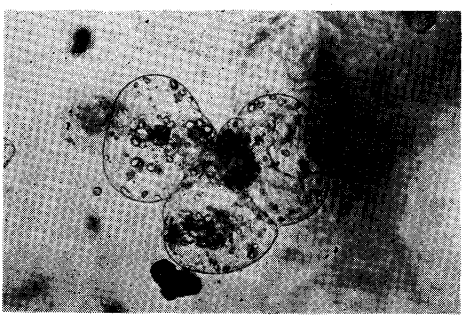

Fig. 2. First cell division in *Luffa cylindrica* protoplasts cultured for 5 days.

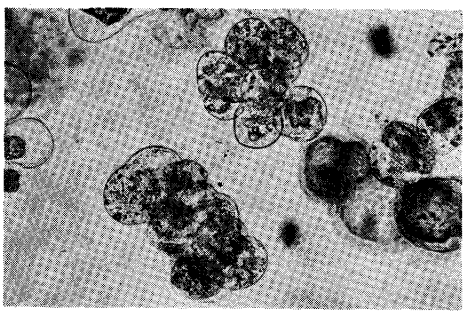

Fig. 3. Cell cluster formed after 15 days of culture.

medium stabilized with 0.25 M mannitol, however, we tested various osmotica at 0.25 M. Galactose and glucose gave higher division rate than mannitol and sorbitol, whereas no division occurred in lactose and sucrose. The best results (21% division) were obtained with galactose. The beneficial effect of galactose as the osmoticum for protoplast culture has not been reported.

The plating efficiency of *Luffa cylindrica* protoplasts was improved further by using 0.5 mg/*l* BA instead of kinetin. Under these conditions, the first division occurred within 5-7 days (**Fig. 2**), and after 10 days about 35% of protoplasts divided at leaset once. Clusters of several cells (**Fig. 3**) were formed from about 1/3 of the divided protoplasts, and these developed later into visible colonies. Attempts to induce differentiation in the protoplast-derived cell lines have so far been unsuccessful.

References

1) Coutts, R. H. A., K. R. Wood, 1977. Plant Sci. Lett., **9**: 45-51.
2) Moreno, V., L. Zubeldia, L. A. Roig, 1984. Plant Sci. Lett., **34**: 195-201.
3) Orczyk, W., S. Malepszy, 1985. Plant Cell Rep., **4**: 269-273.
4) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-479.
5) Kouider, M., R. Hauptmann, J. M. Widholm, R. M. Skirvin, S. S. Korban, 1984. Plant Cell Rep., **3**: 142-145.
6) Maeda, Y., Y. Fujita, Y. Yamada, 1983. Plant Cell Rep., **2**: 179-182.
7) Nakagawa, H., H. Tanaka, T. Oba, N. Ogura, M. Iizuka, 1985. Plant Cell Rep., **4**: 148-150.

≪和文要旨≫

ヘチマ培養細胞プロトプラストの単離と培養

松本省吾**，伊藤三明**，小西宏明**，建部　到*

* 名古屋大学理学部
** 日本メナード化粧品株式会社

ヘチマ培養細胞からプロトプラストを単離し，培養した．浸透圧調節剤を単離の際の 0.4 M マンニトールから，培養時には 0.25 M ガラクトースに変え，さらにサイトカイニンとして BA を用いることにより，Murashige and Skoog を基本とする培地で，約35%のプロトプラストが分裂し，その一部はコロニーを形成した．