Fusion of rapeseed and soybean protoplasts and subsequent division of heterokaryocytes

K. K. KARTHA, O. L. GAMBORG, F. CONSTABEL, AND K. N. KAO
Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan S7N 0W9
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Protoplasts from mesophyll cells of rapeseed plants (Brassica napus L. cv. Zephyr) and cell cultures of soybean (Glycine max (L.) Merr. cv. Mandarin) were induced to fuse and form heterokaryons by using polyethylene glycol. Plastids served as markers to identify heteroplastic fusion products: the heterokaryons shared the chloroplasts from rapeseed and dense cytoplasm and colorless plastids from the soybean protoplasts. About 19–20% of the total population of viable protoplasts were identified as heterokaryons, some of which divided and formed colonies consisting of up to 10 cells within 10–12 days of culturing.

INTRODUCTION

In recent years many attempts have been made to achieve somatic cell hybridization by protoplast fusion (1–4). Recently a procedure was developed that facilitated high-frequency intergeneric fusion of plant protoplasts (5). In this communication we report on the application of this technique to the fusion of mesophyll protoplasts of rapeseed with soybean protoplasts derived from cell suspension cultures, and on subsequent division of the fusion products.

MATERIALS AND METHODS

Protoplasts were isolated from the mesophyll cells of 1-month-old rapeseed (Brassica napus L. cv. Zephyr) plants according to the procedure reported previously (6). The protoplasts of soybean (Glycine max (L.) Merr. cv. Mandarin) were isolated from 2-day-old cell suspension cultures, subcultured every 3 days, by similar methods reported for carrot and Ammi visnaga (7).

After isolation, the rapeseed and soybean protoplasts were washed free of enzymes in a solution containing 0.25 M each of sorbitol and mannitol at pH 5.8 and were resuspended in the same solution keeping about 10⁴ protoplasts/mL. The two kinds of protoplasts were finally mixed in a 1:1 proportion (v:v) and placed in droplets of 100 μl in 60 x 15 mm plastic petri dishes each containing six drops of the protoplast suspension. The protoplasts settled to the bottom of the petri dish within 5–8 min.

The fusion-inducing agent used was polyethylene glycol (PEG, pharmaceutical grade) (5). Five grams of PEG (mol wt 1540) were dissolved in 10 ml of distilled water containing 10 mM CaCl₂·2H₂O, 0.7 mM KH₂PO₄, and 0.1 M glucose (5). The pH of the PEG solution was adjusted to 5.8 with 0.2 N KOH/HCl. When the protoplasts had settled to the bottom of the petri dish, three droplets of about 50 μl of the PEG solution were carefully added to the periphery of each droplet of protoplast suspension. This facilitated an even coverage of protoplast suspension by the PEG solution. After 30 min incubation at 24–26°C, the PEG solution was gradually diluted by adding 2 ml of the rinse medium containing 0.25 M each of sorbitol and mannitol (pH 5.8) twice at 5-min intervals. The rinse medium was finally removed and the protoplasts were washed three times by gradually adding and removing the culture medium (6). Finally 1 ml of the culture medium was added to each dish and the dishes were sealed with Parafilm® and incubated at 26°C at a light intensity of 100 lx over a photoperiod of 16/8 h (6),
Results and Discussion

The mesophyll protoplasts of rapeseed plants contained chloroplasts lining the periphery (Fig. 1), while those from soybean cell cultures contained dense cytoplasm, amyloplasts, and undifferentiated colorless plastids, which have never been shown to synthesize chlorophyll under these conditions (Fig. 2). The plastids were used as markers to identify the heteroplasmic fusion products.

Very tight agglutination occurred between protoplasts immediately after addition of PEG (Fig. 3), and the protoplasts adhered firmly to the bottom of the petri dish. The aggregates consisted of rapeseed + soybean, rapeseed + rapeseed, and soybean + soybean protoplasts. The protoplast aggregates often involved agglutination of many protoplasts, but some aggregates consisted of only 2 protoplasts, either homoplasmic or heteroplasmic aggregates. However, in the presence of PEG, the protoplasts appeared 'distorted' (Fig. 3). The fusion occurred after the gradual dilution of the PEG. Abrupt dilution caused death of protoplasts (5). After the dilution of PEG, the protoplast aggregates returned to their normal shape (Fig. 4). The heterokaryons were easily distinguishable, since they shared the chloroplasts from one partner and dense cytoplasm and plastids from the other (Fig. 4). The heterokaryons assumed various shapes from round to oval or oblong.

On the dilution of the PEG, most of the unfused rapeseed mesophyll protoplasts degenerated over a period of 24 h. However, the unfused soybean protoplasts remained viable and divided within 24 h. Thus the soybean protoplasts originating from cell suspension cultures were able to withstand the PEG treatment, whereas those from the mesophyll cells did not. Immediately after the PEG had been diluted, 19–20% of the total surviving protoplasts were identified as heterokaryons. After 16 h, 9% of the surviving protoplasts were heterokaryons, which turned oval in shape within 48 h, indicative of cell wall regeneration. The first divisions of the heterokaryons (Fig. 5) began within 72 h and the second divisions took place within 5–6 days (Fig. 6). Small colonies consisting of up to 10 cells were formed within 10–12 days. Experiments are underway to study the behavior of nuclei in the fused cells and also to isolate callus arising from the heterokaryons.

Plant regeneration from mesophyll protoplasts of rapeseed has already been achieved (6). The feasibility of producing somatic hybrid plants will now have to be investigated.

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