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PROLIFERATION OF ISOLATED *ACER PSEUDOPLATANUS* EMBRYOS

Isolated embryos of many plants are capable of growing in vitro and developing into normal plants (Rappaport, 1954). In certain cases undifferentiated callus arises, due to cell proliferation on some parts of the embryo. The callus may be maintained in permanent culture by regular transplantations (Curtis et al., 1963; Morel, 1956 Carev et al., 1958; Hildebrandt et al., 1963). The present paper describes some experiments on the cultivation of *Acer pseudoplatanus* embryos and conditions for inducing growth of callus, suitable for further transplantation. Steward et al. (1951) showed that proliferation of potato tuber tissue required the presence of two growth factors — an auxin and a cytokinin from coconut milk. It seems to be generally recognized today, that the presence of an auxin-like and a kinin-like substance is the necessary requirement for both division and growth of plant cells (Kefford et al., 1961; Nitsch, 1963). Results obtained in this work are in agreement with such conceptions.

MATERIAL AND METHODS

Fruits from a Maple tree were collected during June and July 1963 and 1964. In that period embryos were not completely developed. They were 3—10 mm long and all embryonic organs were clearly discernible. The »wings« of the fruits were removed and the remaining part was surface sterilized by 5% sodium hypochlorite solution for two hours. They were then washed with sterile water, opened by a sharp knife and undamaged seeds put into sterile Petri dishes. The seed coat was cut through and the dark green embryo, lying in a droplet of liquid endosperm, removed out of the seed and transferred on a nutrient medium. In some cases embryos were transversally divided into four parts and each part was cultivated separately.

Embryos were grown in test tubes or Erlenmayer flasks, on a basal medium containing White's (1943) or Heller's (1953) mineral solution, 2% succrose and 0,8% agar. To this medium following natural or synthetic growth factors were added in different combinations: coconut milk, yeast extract, casein hydrolysate, a mixture of vitamins, adenin, kinetin, auxins and gibberellic acid. Embryo cultures were maintained in a temperature controlled room at 25° C, in the diffuse daylight, not exceeding 300 lux in intensity.

RESULTS

Three to four weeks after planting the embryos on the nutrient medium, swelling of the hypocotyls could be observed, which later formed calluses of different size. In the cultures with embryos cut in parts, it was ascertained that a callus developed only from the hypocotyl cells. Parts consisting of root apex, upper end of the hypocotyl with the bud, or cotyledons never developed callus.

Experiments with more than 1000 embryos grown on different nutrient media, showed that a callus may be obtained only if the medium was supplemented by coconut milk and auxins. Fig. 1 represents the synergistic effect of coconut milk and α-naphtylacetic acid (NAA) or 2,4-dichloro-phenoxyacetic acid (2,4-D) in the stimulation of callus growth. It may be seen that coconut milk alone did not stimulate the proliferation of the cells; NAA alone provoked a swelling of the hypocotyl, while a voluminous callus developed if both substances were present. The best results were obtained by using 15% coconut milk and NAA in concentration of 3 mg/l. 2,4-D and IAA (the later not shown in the picture) also stimulated callus growth, but in much less extent.

Embryos cultivated on other nutrient media sometimes developed small calluses, which stopped growing soon and were not suitable for

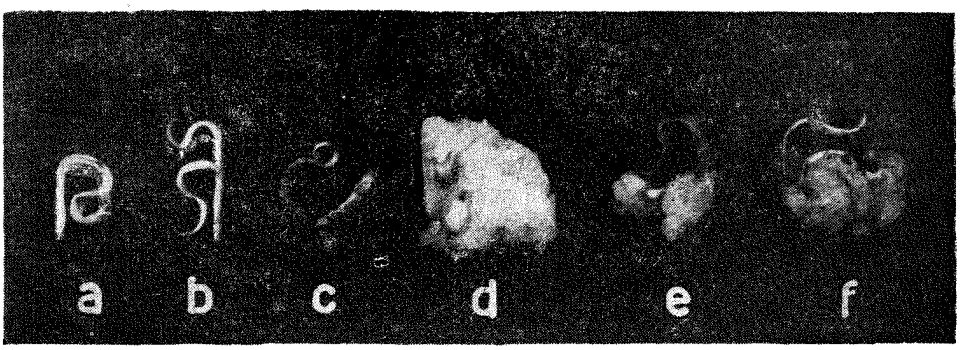

Fig. 1. Proliferation of *Acer pseudoplatanus* embryos

(a) Basal medium (BM), containing White's mineral solution, 2% succrose and 0,8% agar; (b) BM + 15% coconut milk; (c) BM + 3 mg/1 NAA; (d) BM + 15% coconut milk + 3 mg/1 NAA; (e) BM + 15% coconut milk + 1 mg/1 2,4—D; (f) BM + 15% coconut milk + 5 mg/1 2,4—D. Photograph taken after two months.

subculturing. Coconut milk could not have been replaced by 1,5% yeast extract, 0,5% casein hydrolysate, kinetin or adenin. Vitamin mixture (Henderson et al., 1952) alone or combined with other substances did not contribute to callus formation. No effect of gibberellic acid was observed either. Finally, a number of embryos were cultivated on a fully synthetic medium, claimed by Koblitz (1962) to substitute efficiently for coconut milk in tissue cultures of *Daucus carotta,* but without positive results. This medium, however, did stimulate the normal growth of young plantlets.

Both mineral solutions tried (White's and Heller's) were suitable for the formation of callus. White's solution was chosen for further work, because it caused less lignification of cell walls.

It was attempted in a series of experiments to prevent the destruction of chlorophyll by growing embryos in the light of higher intensity. Those cultures were maintained in the strong daylight near a northern window. As it is shown in Fig. 2, strong daylight inhibited markedly the growth of the callus, but the greening was not observed.

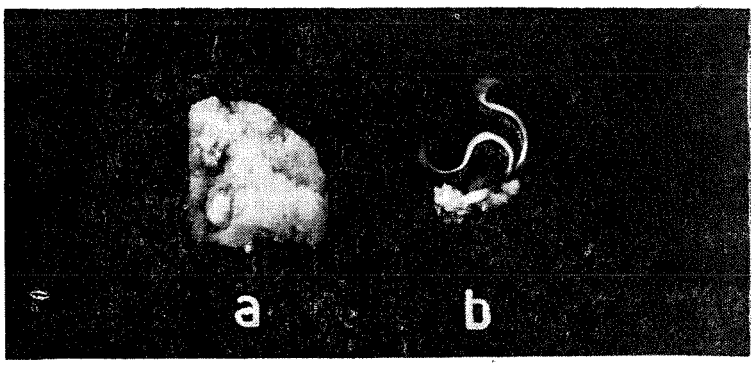

Fig. 2. Influence of light on the proliferation of embryos; nutrient medium as in (d), Fig. 1

(a) embryo grown in the weak daylight (less than 300 lux); (b) embryo grown in the strong daylight near a northern window. Photograph taken after two months.

A callus produced by proliferation of hypocotyl cells may be designated as a »dissociated type callus« (Gautheret, 1959). Separate groups of cells were loosely bound together and the tissue could be easily macerated into single cells or small clusters of cells by transferring it into a liquid medium. Three types of cells were osberved under the microscope: (a) isodiametric, about 55 microns in diameter; (b) elongated cells, about 320 × 18 microns, and (c) an intermediary type, about 85 × 55 microns in size.

A callus developed on a suitable medium may be subcultured successfully for a longer time. Further work on the conditions required for unlimited growth of the callus is in progress.

SUMMARY

1. Isolated embryos of *Acer pseudoplatanus* may under favourable conditions give rise to a callus, produced by proliferation of hypocotyl cells. The callus could be grown in vitro for a longer time.

2. Two types of growth factors were necessary for the formation of the callus. They were coconut milk and synthetic auxins. A significant synergism between 15% coconut milk and 3 mg/l NAA was shown to exist.

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Rezime

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PROLIFERACIJA IZOLOVANIH EMBRIONA *ACER PSEUDOPLATANUS*

1. Izolovani embrioni *Acer pseudoplatanus* mogu pod povoljnim uslovima da razviju kalus, zahvaljujući proliferaciji ćelija hipokotila. Kalus može duže vreme da se gaji in vitro.

2. Za formiranje kalusa su neophodne dve vrste faktora rastenja. To su kokosovo mleko i sintetički auksini. Zapaženo je da postoji značajna sinergija između 15% kokosovog mleka i 3 mg/l NAA.