Initiation and proliferation of carrot callus using a combination of antibiotics

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Abstract. Calli were initiated from carrot (Daucus carota L. subsp. sativus Hoffm.) root explants and grown on media supplemented with a combination of carbenicillin at 0.3 mg/ml and vancomycin at 0.1 mg/ml in the absence of plant hormones or hormone analogs. The growth rate was about half of that obtained with a combination of α-naphthaleneacetic acid and N6-benzyladenine at 1 mg/l each. Carbenicillin in the combination with vancomycin could be replaced by penicillin G; other penicillins tested in this combination, however, caused only limited growth. The calli produced can be grown on media supplemented with α-naphthaleneacetic acid and N6-benzyladenine, but not on media without the antibiotics and the plant growth substances. Calli obtained using the plant growth substances can also be subcultured on media supplemented with only the antibiotics.

Key words: Antibiotics and callus culture – Callus (cultured with antibiotics) – Daucus (callus growth with antibiotics)

Introduction

Initiation and proliferation of plant callus in vitro normally require the presence of a plant hormone or plant-hormone analog, or a combination of such growth substances in the growth media. Auxins, cytokinins and some compounds with auxin or cytokinin activity have been effective in most cases (for reviews, see Gresshoff 1978; Krikorian et al. 1987). The requirement for cytokinins may be satisfied by using other types of compounds: e.g. two dehydrodiconiferyl glucosides in micromolar concentrations can replace cytokinins to stimulate the growth of tobacco pith tissue in the presence of α-naphthaleneacetic acid (α-NAA) (Binns et al. 1987).

Camus and Lance (1955) were the first to report that the growth of normal plant tissues in vitro can be stimulated by an antibiotic. They obtained an increased fresh weight (about threefold) of auxin-requiring artichoke tuber tissues after an incubation period of about six weeks by using penicillin G or procain penicillin in the absence of auxin, although the effect was suspected by others to be caused by traces of auxin in the antibiotic samples used (Brian 1957). These two authors, however, were not able to obtain this stimulation on carrot root tissues under the same condition. We report here that calli can be initiated from carrot root tissues and subcultured using a combination of a penicillin, either carbenicillin or penicillin G, and vancomycin, in submicromolar concentrations, in place of plant growth substances.

Material and methods

Carrot (Daucus carota L. subsp. sativus Hoffm.) roots were purchased from local stores. They were washed with a detergent solution, peeled, and sterilized with 1.0% NaOCl (made from commercial bleach) for 15–20 min. After several rinses with sterile water, the top halves of the carrots were cut transversely into approx. 5-mm-thick slices. Each slice was further cut into four equal sections, and a wedge of tissue approx. 2.5 mm wide and deep was then cut from the cambial-ring area (Fig. 1). Thus, four explants were obtained from each slice. Twelve pieces were weighed and incubated on 50 ml of semisolid growth media without or with various supplements in 125-ml flasks at 25°C under continuous light from fluorescent lamps (daylight, 40 W; Sylvania, Danvers, Mass., USA) separated by a distance of 10 cm and about 40 cm above the cultures. For subculturing, callus tissues were cut into small pieces, each weighing about 10 mg. Twelve pieces were weighed (between 150 and 250 mg) and incubated on 50 ml of semisolid growth medium under the same light and temperature conditions.

Two media were used. Both contained Murashige and Skoog's (1962) macro- and micronutrients. Medium A (Chang et al. 1983) contained the following additions (in mg/l): myo-inositol (200), glycine (6), t-glutamic acid (50), L-asparagine (50), t-glutamine (100), L-asparagine (25), thiamine (1), pyridoxal · HCl (1), choline chloride (1), calcium pantothenate (1), nicotinamide (1), folie acid (1), nicotinic acid (0.4), riboflavin (0.1), adenine sulfate (5), urea (30)
and 30 g/l sucrose. The second medium was the basic “revised” medium of Murashige and Skoog (1962, Table 6) which contains glycine (2), nicotinic acid (0.5), pyridoxine·HCl (0.5), thiamine (0.1), myo-inositol (100) and 30 g/l sucrose in addition to the macro- and micronutrients. The media were sterilized at 121°C for 15 min and solidified using 0.8% Bacto agar. N6-Benzyladenine (BA), α-NAA and antibiotics added as supplements were filter-sterilized before they were added to the sterilized medium; BA and α-NAA were added to the medium at the concentration of 1 mg/l each. Tissues grown on the α-NAA–BA medium were transferred to new media after four weeks of incubation; because of their less rapid growth, antibiotic-grown calli were transferred on the fifth or sixth week.

Ampicillin, carbenicillin, epicillin, metampicillin, benzylpenicillin, piperacillin, cephalexin, vancomycin, α-NAA and BA were all obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Results

Effects of carbenicillin and vancomycin on the growth of primary explants. In the course of studies on plant transformation, primary explants of carrot roots were found to proliferate in the absence of growth substances on Medium A supplemented with a combination of carbenicillin at 0.3 mg/ml and vancomycin at 0.1–0.2 mg/ml (Fig. 1). The synergistic effect of carbenicillin and vancomycin is demonstrated in Fig. 2. In the presence of 0.3 mg/ml of carbenicillin or 0.2 mg/ml of vancomycin alone, the tissues showed very little growth (1 and 2, respectively). A combination of 0.05 mg/ml of carbenicillin and 0.05 mg/ml of vancomycin produced an enhancement of growth (3). A further increase of carbenicillin concentration to 0.1 mg/ml and 0.3 mg/ml in the combination further increased the growth of the tissues (4 and 5, respectively), and so did an increase of vancomycin concentration from 0.05 to 0.1 mg/ml at 0.3 mg/ml of carbenicillin. However, raising the vancomycin concentration from 0.1 to 0.2 mg/ml did not promote growth further (data not shown).

The effects of other penicillins in combination with