Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):39-40, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Microalgal and cyanobacterial extracts in the tissue cultures of higher plants (pea, tobacco, beet)

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ABSTRACT The empirical approach that has been extensively used in studies on *in vitro* organogenesis has shown that success is largely dependent on three factors: explant choice, medium composition, and control of the physical environment. Manipulation of these factors leads to the initiation of organized development. It is well known that the concentration and combination of growth regulators govern plant regeneration. There is accumulating evidence that some of the hormones which operate in higher plants could have similar roles in algae, even in microalgae and cyanobacteria. The in vitro culture of recalcitrant plants (such as pea, beet) needs other organic growth substances than plant hormones. In this study we have evaluated the beneficial effects of some extracellular compounds derived from axenic cultures of microalgae. The dilution of freeze-dried biomass from some microalgae and cyanobacteria could be useful for the improvement of *in vitro* culture media of economically important crops.
Acta Biol Szeged 49(1-2):39-40 (2005)

KEY WORDS

microalgal extracts
in vitro culture
Pisum sativum L.
Nicotiana tabacum L.
Beta vulgaris L.

Plant tissue culture can be considered to involve three phases: first, the isolation of plant tissue from its usual environment; second, the use of aseptic techniques to obtain clean material free of the usual bacterial, fungal, viral, and even algal contaminants; and third, the culture and maintenance of this material *in vitro* in a strictly controlled physical and chemical environment (Hall 1999). The components of this environment are then in the hands of the researcher, who gains a considerable degree of external control over the subsequent fate of the plant material concerned. An extra, fourth phase may also be considered where recovery of whole plants for rooting and transfer to soil is the ultimate goal. The empirical approach that has been extensively used in studies on *in vitro* organogenesis has shown that success is largely dependent on three factors: explant choice, medium composition, and control of the physical environment. It is well known that the concentration and combination of growth regulators govern plant regeneration. Many experimental results suggest the plant growth regulator (PGR) content and its physiological function in macro- and microscopic algae (Jameson 1993; Stirk et al. 2002; Ördög et al. 2004). Various compounds of microalgae could be useful sources to enhance or substitute the influence of synthetic PGRs on tissue cultures of different plants *in vitro*.

Several strains of Mosonmagyaróvár Microalgal Collection (MACC) have been tested as a source of growth regulators for *in vitro* cultures of pea (*Pisum sativum* L.), tobacco (*Nicotiana tabacum* L.) and beet (*Beta vulgaris* L.).

Materials and Methods

Algal cultures: the batch cultures of microalgae and cyanobacteria (Table 1.) were grown in axenic conditions in an apparatus for laboratory algal bioassay described by Ördög (1982). The cultures were incubated at 25°C, in a 12:12 h light-and-dark cycle. They were aerated with 20 L h^{-1} compressed air (in the light period enriched with 1.5% CO_2) through sterile cotton filters and mixed manually twice a day. The algae were harvested in the early stationary phase of growth by centrifugation for 15 min.

Plant material: pea (cv. 'Akt'), tobacco (cv. 'SR-1') and beet seedlings (cv. 'Aranymono') were grown in aseptic conditions on Knop (1865) medium solidified with 6 g L^{-1} agar. Shoots (plus stem and mesocotyl segments in pea) were excised from 10-day-old seedlings.

Culture media: after centrifugation the supernatant of microalgal cultures were used to dilute the stock solutions of B5 medium instead of distilled water. A combination of benzylamino purine (BAP) and naphthalene acetic acid (NAA), 4,5 mg L^{-1} and 0,02 mg L^{-1}, respectively, were used. Hormone-free medium served as control. The combined effect of microalgal supernatants and synthetic growth regulators was also studied. The freeze-dried biomass (2 g L^{-1} and different dilutions: 0.2 - 0.6 - 1 - 2 g L^{-1}) of strains MACC-203, -304, -533, -612 was also used in comparison with synthetic PGRs (BAP and NAA).

In vitro cultures: ten shoots (plus stem and mesocotyl segments in pea) were placed in a 9 cm Petri dish containing the B5 culture media solidified with 7 g L^{-1} agar. The cultures were incubated at 25°C, in a 16:8 h light-and-dark cycle. Fresh weight of tissues, the number of regenerated shoots,

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Table 1. Microalgal and cyanobacterial strains tested in our experiments.

Microalgal strains	Species
MACC-33	*Chlorococcum ellipsoideum* Archib. et Bold
MACC-44	*Scotiellopsis terrestris* (Reisigl) Punc. et Kal.
MACC-67	*Selenastrum rinoi* Kom. et Comas
MACC-101	*Synechococcus sp.*
MACC-114	*Scenedesmus obliquus* (Turp.) Kütz.
MACC-137	*Scotiella nivalis* (Schuttl.) Fritsch
MACC-330	*Chlorococcum humicolum*
MACC-438	*Chlorella sp.*
MACC-696	*Chlorococcum sp.*
Cyanobacterial strains	Species
MACC-203	*Pseudochlorococcum typicum*
MACC-304	*Anabaena sphaerica*
MACC-533	*Coenochloris sp.*
MACC-612	*Nostoc entophytum*

leaves and roots after eight weeks of culture. Data were analyzed with one way ANOVA.

Results and Disussion

Brown and compact calli were developed on media supplemented with only the supernatant of microalgal strains in pea mesocotyl cultures, while green calli and small shoots were obtained with combination of synthetic PGRs. The combination of extracellular compounds from microalgae and synthetic PGRs produced more fresh weight and regenerated shoot numbers than both controls in all cultures. In shoot cultures the medium with 2 g L^{-1} freeze-dried (f.d.) biomass was found better than hormon-free B5 medium, thus it did not substitute the synthetic plant hormones. We could also state that the concentration range 0.2-0.6 g L^{-1} from MACC-304 and 1 g L^{-1} from MACC-612 f.d. biomass have beneficial effects on *in vitro* cultures of pea. In stem segment cultures a growth inhibition effect of microalgae and cyanobacteria was occurred. No multiple shoot regeneration, although one more viable shoot was developed in mesocotyl cultures.

In tobacco cultures shoots had increased fresh weight compared to both controls on culture medium supplemented with 2 g L^{-1} f.d. biomass of MACC-304, MACC-612. Compounds from these cyanobacteria have beneficial effect on leaf development. The dilutions of f.d. biomass (0.2 g L^{-1} from MACC-304 and 1 g L^{-1} from MACC-612) have produced more vigorous cultures than the controls. A stimulation of root development was recorded in some cases.

Supernatants of strains MACC-33 and MACC-67 served as a very good supplement for shoot cultures of beets even used them alone or combined with BAP and NAA. Vigorously grown shoots, sometimes with roots, were developed on these cultures.

Complex nutritive mixtures have been added to plant tissue culture media in the past decades. Nowadays media containing only chemically-defined compounds are commonly used (Hall 1999). The *in vitro* culture of recalcitrant plants (such as pea, beet) needs other organic growth substances than plant hormones. In this study we have evaluated the beneficial effects of some extracellular compounds derived from axenic cultures of microalgae. Supernatants from cultured microalgae, as an 'unknown organic mixture', have improved the regenerated shoot numbers on media suplemented with other plant hormones. The dilution of freeze-dried biomass from some microalgae and cyanobacteria could be useful for the improvement of *in vitro* culture media of economically important crops.

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