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The effect of cyanobacterial compounds on the organogenesis of pea cultured *in vitro*

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ABSTRACT Many experimental results suggest the plant growth regulator (PGR) content and its physiological function in macro- and microscopic algae. Various compounds of cyanobacteria could be useful sources to enhance or substitute the influence of synthetic PGRs on tissue cultures of recalcitrant plants in vitro. In this study we have evaluated the beneficial effects of some extracellular compounds derived from axenic cultures of cyanobacteria. The cyanobacterial compounds in biomass alone have produced lower rates of shoot regeneration and gained smaller fresh weights compared to the PGRs control. They are not like real substitutes of synthetic PGRs but as a supplement in culture media resulting more vigorious cultures and regenerated shoots.
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KEY WORDS

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The techniques of *in vitro* cell and tissue cultures originally were elaborated to study plant nutrition and morphogenesis. Recently the use of regenerants derived from these cultures became almost the basic practical approach of plant biotechnology. Old and new methods in tissue cultures have based on three factors, namely: 1. isolation of explants (resulting a discontinuation among cells, tissues and organs compared to the intact plant), 2. to sustain these isolates in controlled conditions, that means aseptic terms of culturing which can be the third factor. The chemical composition of culture media and physical parameters of aseptic cultures have effects on the expression of genotype and phenotype potential of explants. *In vitro* organogenesis means the changes resulting a unipolar tissue primordium from isolated cells and tissues. Small size of precursor cell populations (meristemoids) have to be emerged before coming out these structures. Competent cells of isolates can develop to meristemoids via direct or indirect way, that means through a callus phase. Plant hormones have central role in the control of *in vitro* organogenesis. Auxins and some cytokinins have crucial role in gene expression during plant development *in vitro*. Natural compounds derived from different plant materials have several effects on regeneration of recalcitrant plants, such as grain legumes. These chemicals can be a kind of substitution of synthetic growth regulators. Recent knowledges in microalgal biotechnology state that plant growth regulators (PGRs) are present in macro and microalgae, too (Jameson 1993; Stirk et al. 2002; Ördög et al. 2004). The biomass of some cyanobacterial strains were used in tissue cultures of pea (*Pisum sativum* L.).

Materials and Methods

Plant material: biomass derived from four strains of our microalgal and cyanobacterial collection (MACC) (MACC-203 - Pseudochlorococcum typicum, MACC-304 - Anabaena sphaerica, MACC-533 - Coenochloris sp., MACC-612 - Nostoc entophytum) have been tested as a source of growth regulators for *in vitro* cultures of *Pisum sativum* L. cv. 'Akt', in three different harvest dates.

Explants: shoot tips and mesocotyl segment of *in vitro* grown 7-days-old seedlings of pea were cultured on B5 medium (Gamborg et al. 1968). The centrifuged and freeze-dried biomass of aseptically grown cyanobacteria were added to culture media first in 2 g/l concentration. Afterwards two chosen strains (MACC-304 and -612) were tested in different dilutions (0.2 - 0.6 - 1 - 2 g/l).

Culture media: a combination of benzylamino purine (BAP, 4.5 mg/l) and naphthalene acetic acid (NAA, 0.02 mg/l) (B5NB) plus a hormon-free medium (B50) was used as controls. The combined effect of microalgal supernatants and synthetic growth regulators was also studied.

Evaluation: fresh weight of tissues, the number of regenerated shoots and leaves were scored after eight weeks of culture. Data were analyzed with one way ANOVA.

Results and Discussion

Plant growth regulators derived from 2 g/l biomass of cyanobacteria showed positive effects on tissue cultures in our earlier experiments. Based on these findings cyanobacterial strains MACC-304 and -612 were found better than others. Highest fresh weight were scored at 0.2-0.6 g/l biomass supplements in the tested concentration range (0.2 - 0.6 - 1 - 2 g/l). Shoot proliferation was more intense in mesocotyl segments compared to shoot tips. Lower shoot regeneration rates and fresh weights were obtained in culture media supplemented with only the cyanobacterial biomass.

Complex nutritive mixtures have been added to plant tissue culture media in the past decades. Nowadays media

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containing only chemically-defined compounds are commonly used. The *in vitro* culture of recalcitrant plants (such as pea) 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 cyanobacteria.

After the results of our previous bioassays 2 g/l biomass of MACC-304 and MACC-612 were found beneficial for the *in vitro* growth of tissues from pea. The dilutions of biomass (0.2 – 0.6 – 1 – 2 g/l) have increased the fresh weight of cultures. The optimal concentration ranged from 0.2 to 0.6 g/l. Mesocotyl segments served better sources for the shoot regeneration. The cyanobacterial compounds in biomass alone have produced lower rates of shoot regeneration and gained smaller fresh weights compared to the PGRs control. They are not like real substitutes of synthetic PGRs but as a supplement in culture media resulting more vigorious cultures and regenerated shoots.

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