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Transitory Pulse-Like Treatment with IAA Solution More Effectively Induces Xylogenesis in Callus Culture than Permanent Presence of the Auxin

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Abstract. Differentiation of *Acer pseudoplatanus* L. cells into tracheary elements (TE) and an increase in the number of TE in *Haplopappus gracilis* (Nutt.) A. Gray calli were observed after pulse-treatment of the cultures grown in glass tubes with indole-3-acetic acid (IAA) solutions. The effect was enhanced if the treatment was repeated in three subsequent days. The manner of IAA application which caused a wave-like pattern of IAA flow through the callus culture appeared to be more important than the IAA concentration. Induction of differentiation of *A. pseudoplatanus* cells and an increase in the number of TE in *H. gracilis* callus did not occur when the calli were grown on agar media supplemented with increased concentrations of IAA.

Studies concerning the distribution of plant hormones in whole plants have shown that IAA concentration does not decrease linearly, but changes periodically in a wave-like manner along a stem and a root (Zajączkowski and Wodzicki 1978a,b, Wodzicki and Wodzicki 1981). Some authors (Zajączkowski *et al.* 1983) suggested that this phenomenon might play an important role in plant morphogenesis and cellular differentiation. Research carried out on animals and animal cells in vitro has shown that hormones (and other substances) which induce cellular differentiation need not to act permanently, and their short lasting contact with cells is sufficient to induce the effect (Puro and Agardh, 1984, Chiang *et al.* 1987, Reiss and Korohoda, 1988). Research concerning the effect of plant hormones on plant cell growth and differentiation *in vitro* has been carried out commonly under conditions when the tested substance was continuously present in the culture medium. The aim of the experiments described in this paper was, to examine whether or not the pulse-like treatment of plant tissue with auxins can induce cellular differentiation.

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MATERIAL AND METHODS

Established callus lines of two plants, *Haplopappus gracilis* (NUTT.) A. GRAY (*Compositae*) and *Acer pseudoplatanus* L. (*Aceraceae*) "white" line, were used in the experiments. Calli were cultured in glass tubes of 5 mm in diameter and 40 mm in length (Fig. 2). Filter paper was placed at one end of the tube. Small pieces of calli were inserted into the tube and placed on the filter paper which was then immersed in a liquid nutrient medium in an Erlenmeyer flask. Nutrient media were prepared according to Eriksson (1965) for *H. gracilis,* and according to Bligny (1977) for *A. pseudoplatanus.* The callus of *H. gracilis* was grown in the dark at 27 ± 1 °C, whereas that of *A. pseudoplatanus* was grown in the light at the same temperature.

The culture conditions described above were used in parallel with control cultures grown on agar media. Fresh mass increment was determined and calculated as described by Kuternozińska *et al.* (1988). Total protein content was measured according to the method of Lowry in calli grown under both culture conditions.

Effects of IAA were tested on calli which had been grown for about three weeks in glass tubes. The concentration of IAA in the control, nutrient media was 1 mg l^{-1}. The tested IAA solution (10 mg l^{-1} in nutrient medium) was applied as drops on top of the calli every ten minutes for two hours. Then the calli were washed with control media (with 1 mg l^{-1} of IAA) and the nutrient media in Erlenmeyer flasks were changed to maintain the control concentration

TABLE 1

Fresh mass increment in callus cultures from two plants (*Haplopappus gracilis* and *Acer pseudoplatanus*) grown on agar medium and in glass tubes. Callus of *H. gracilis* was cultured in the dark and callus of *A. pseudoplatanus* in the light, both at 27 ± 1 °C. Given data represent mean averages from 7 to 10 replicates. Fresh mass increment was expressed as a difference between the fresh mass of the callus cultured for an indicated number of days minus fresh mass of callus plated at the beginning of the experiment divided by the fresh mass of the transplanted callus

	H. gracilis		*A. pseudoplatanus*	
	Fresh mass increment [mg mg^{-1}]			
Day of culture	on agar medium	in glass tubes	on agar medium	in glass tubes
6	0.59 ± 0.14	0.29 ± 0.15	1.58 ± 0.30	0.00
9	n.m.*	n.m.	1.80 ± 0.40	0.00
13	n.m.	n.m.	6.80 ± 0.40	1.70 ± 0.10
16	2.32 ± 0.37	0.74 ± 0.31	10.50 ± 2.60	1.80 ± 0.20
21	3.97 ± 1.08	1.28 ± 0.96	28.20 ± 3.00	2.20 ± 0.10

*) n.m. – not measured

TABLE 2

Total protein content in callus cultures of *H. gracilis* and *A. pseudoplatanus* grown on agar medium and in glass tubes, under conditions described in Table 1. The number shown represent mean averages of 3 replicates

Day of culture	*H. gracilis*		*A. pseudoplatanus*	
	Total protein content [mg 100 mg^{-1} (fresh mass)] of calli grown:			
	on agar medium	in glass tubes	on agar medium	in glass tubes
3	0.82 ± 0.08	0.89 ± 0.04	0.45 ± 0.04	0.44 ± 0.08
7	0.85 ± 0.18	0.96 ± 0.06	0.58 ± 0.16	0.49 ± 0.10
11	1.10 ± 0.08	0.98 ± 0.11	0.53 ± 0.05	0.75 ± 0.05
14	0.73 ± 0.26	1.26 ± 0.08	0.58 ± 0.08	0.68 ± 0.12
16	0.63 ± 0.09	1.13 ± 0.10	n.m.	n.m.
18	n.m.	0.88 ± 0.14	0.30 ± 0.06	0.31 ± 0.08

of IAA. The treatment was repeated once daily for 3 subsequent days. The control calli were treated in the same way with the medium containing the control (1 mg l^{-1}) concentration of IAA.

The number of TE in the control and tested cultures was determined with a light contrast phase and polarization microscope 9 d after the last treatment. The number of TE per 100 mg of fresh mass of tissue was estimated in all cultures. The traditional cultures on agar media with an increased concentration of IAA were used as the second control.

RESULTS

In the first set of experiments the growth of calli in glass tubes and on agar media was compared. Fresh mass increment in calli grown in glass tubes was lower than that of calli grown on agar (Table 1). Nevertheless, the total protein content showed less pronounced difference for calli grown in two different conditions (Table 2), though some delay of growth in glass tubes in comparison to growth on agar was visible.

Cells of *Acer pseudoplatanus* callus remained undifferentiated in both types of culture (Fig. 1b) and in the calli of *Haplopappus gracilis* the same number of cells per 100 mg of fresh tissue showed a thickening of secondary walls under both culture conditions (Fig. 1a). The increase in IAA concentration from 1 to 10 mg l^{-1} in agar media did not stimulate xylogenesis in *A. pseudoplatanus* callus cultures and did not change the number of tracheary elements found in *H. gracilis* callus cultures (Fig. 1).

In the following series of experiments, the cultures in glass tubes were treated for three subsequent days with liquid media with an increased concentration

of IAA (10 mg l^{-1}), as described in Material and Methods. In the cultures of *H. gracilis* callus treated in this manner more than three-fold increase in the number of tracheary elements was observed. Differentiated cells showed reticulate or pitted patterns of secondary wall thickening (Fig. 2b).

The treatment of *A. pseudoplatanus* callus cultures in glass tubes with media with increased IAA concentrations induced the appearance of numerous

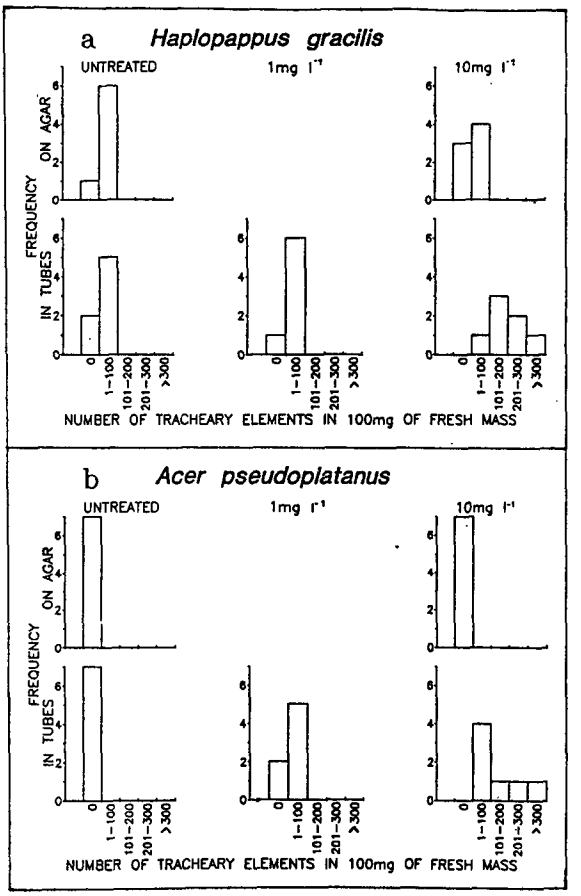

Fig. 1. The effect of pulse-like treatment with the solution supplemented with IAA (10 mg l^{-1}) upon the induction of tracheary elements (TE) in calli of *Haplopappus gracilis* (a) and *Acer pseudoplatanus* (b). *H. gracilis* was grown in the dark at 27 ± 1 °C. *A.pseudoplatanus* was grown in the light at 27 ± 1 °C. The number of TE was estimated in 7 small pieces (8–10 mg) of calli 9 d after the last treatment and then calculated per 100 mg of fresh mass. The number of TE was also estimated in calli grown on agar media with the concentration of IAA the same as in the solution used for temporary treatment of calli grown in glass tubes.

a) *H. gracilis* was grown on agar media (top) or in the glass tubes and treated once daily for two hours during three subsequent days with IAA solutions (bottom),

b) *A.pseudoplatanus* was grown on agar medium (top) or in the glass tubes and treated for two hours during three subsequent days with IAA solutions (bottom).

tracheary elements in the cultures (Fig. 1b). The differentiated cells had a secondary wall thickening mainly of the reticulate type (Fig. 2c).

DISCUSSION

Comparison of the fresh mass increment of *H. gracilis* and *A. pseudoplatanus* calli grown in glass tubes and on agar shows that calli grow more slowly in tubes. Nevertheless, the total protein content for calli grown under both culture conditions was similar. According to Bligny (1977), the total protein content reflects the physiological state of plant cell cultures. Also, microscopic examination of cells grown in glass tubes did not show any signs of cell injury or death. Moreover, in cultures grown under such conditions no increase in the number of TE elements in callus of *H. gracilis* and no induction of differentiation of cells to TE in the cultures of *A. pseudoplatanus* callus were observed.

The temporal pulse-like treatment of cultures with media with the increased IAA concentration proved to be much more effective in induction (in *A. pseudoplatanus*) or stimulation (in *H. gracilis*) of the differentiation of callus cells to tracheary elements than growing cells on agar media in the constant presence of increased auxin concentration. As was described by List (1969) continuously applied IAA caused habituation of the treated tissue and the observed reaction ceased after the several hours of treatment. This might be connected with so far undetermined characteristics of auxin receptors. The response of plant callus cells seems to depend in this case upon a temporal gradient or temporal cell contact with the hormone rather than upon the hormone concentration, if it is continuously present in the culture medium. This conclusion is in accordance with numerous theoretical considerations and with the expected wave -like pattern of auxin flow along plant organs *in vivo* (Hejnowicz and Erickson 1968, Hertel and Flory 1968, Wodzicki and Wodzicki 1981), but here, additionally, it is based on results of experiments carried out under *in vitro* conditions. The cultures of mesophyll cell suspensions in which cell differentiation occurs easily, such as in the case of *Zinnia,* are an excellent object for research concerning the biochemical and genetic mechanisms of cell differentiation (Falconer and Seagull, 1988). On the other hand, the callus cultures in which differentiation does not occur at all under control conditions (as in the callus cultures of *A. pseudoplatanus*) seem to be very suitable for studies concerned with the mechanisms of induction of plant cell differentiation.

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Fig. 2 at the end of the issue

Ranjeva, R., Boudet, A. M. (ed.): Signal Perception and Transduction in Higher Plants. – NATO ASI Series, Series H: Cell Biology. Springer-Verlag, Berlin–Heidelberg 1990, 344 pp. Hardcover DM 178,–

The process of perception and transduction of the signal is of vital importance for both animal plant cells. Plants are immobile and therefore they possess some specific strategies how to resist against significantly changed environment. These strategies involve also signals and modifications in signal transduction system.

This book represents a state-of-the-art overview of the topic in plants. Receptors for auxin and fusicoccin, solubilized receptor reconstitution, the role of ethylene and ATPases activity modulated by various signals are discussed in terms of signal perception. Transduction of signals is presented in context of calcium message, phospholipid derived messengers and phosphorylation/dephosphorylation mechanisms. Also new approaches and complementary disciplines as electrophysiology, cell imaging and gene cloning are included. The publication is concluded by brief review of the present state of knowledge of signal perception and transduction in plants and by suggestions for future development. It is supplemented with valuable subject index.

This volume brings a very informative survey of the topic and seems to be almost necessary for everybody who is interested in the field of plant biochemistry and physiology.

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