Growth responses of excised radish cotyledons to *meta*-topolin

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Palavan-Ünsal, N., Çağ, S. and Çetin, E. 2002. **Growth responses of excised radish cotyledons to *meta*-topolin.** Can. J. Plant Sci. **82**: 191–194. *Meta*-topolin (*m*T) has been established as an active aromatic cytokinin by Strnad et al. (1997). The present investigation assessed the effects of *m*T on radish cotyledon growth. The effect of *m*T on cotyledon growth, chlorophyll, N and soluble protein contents were measured. Peroxidase (POD) activity and polyamine (PA) contents were also determined. Treatment at 0.25, 0.5 and 1.0 mM *m*T concentrations resulted in 44, 50 and 57% increments in cotyledon growth, respectively, compared with control cotyledons. Increase in chlorophyll content per cotyledon was highest at low *m*T concentrations. Peroxidase activity was highest at 1.0 mM *m*T. Soluble protein content was reduced by the application of *m*T.

Key words: Cotyledon growth, *meta*-topolin, polyamine, peroxidase, radish cotyledons

Palavan-Ünsal, N., Çağ, S. et Çetin, E. 2002. **Incidence de la *méta*-topoline sur la croissance des cotylédons de radis excisés**. Can. J. Plant Sci. **82**: 191–194. Strnad et al (1997) avaient démontré que la *méta*-topoline (*m*T) agit comme une cytokinine aromatique. La présente étude devait préciser les effets de la *m*T sur la croissance des cotylédons de radis. Les auteurs ont mesuré l'incidence de la *m*T sur la croissance des cotylédons et la concentration de chlorophylle, d'azote et de protéines solubles. Ils ont aussi déterminé les effets de l'hormone sur l'activité de la peroxydase et la teneur en polyamines. L'application d'une solution de *m*T à 0,25, à 0,5 ou à 1,0 mM, respectivement, accélère la croissance des cotylédons de 44, de 50 et de 57 %, comparativement à celle des cotylédons témoins. La plus forte hausse de la concentration de chlorophylle survient aux concentrations de *m*T le plus faibles. La peroxydase, en revanche, est la plus active avec la solution de *m*T à 1,0 mM. L'application de *m*T réduit la concentration de protéines solubles.

Mots clés: Croissance des cotylédons, *méta*-topoline, polyamine, peroxydase, cotylédons de radis

It is well known that cytokinins manifest a number of common physiological properties: stimulation of cell division, retarding of leaf senescence, inhibition of protease activity, etc. (Letham 1971; Thimann 1980; Durmuş and Kadioğlu 1998).

Bioassays are used to determine the relative biological activity of plant hormones. The cytokinin bioassays used most frequently depend on growth of tissues in sterile culture (Letham 1967). Such methods are extremely sensitive, but the assay time is normally at least 3 wk. Letham (1971) described a rapid bioassay for cytokinins, based on the ability of these compounds to promote markedly the expansion of radish cotyledons excised soon after seed germination. The effects of kinetin, benzyladenine (BA) and its riboside have been documented on radish cotyledons. *Meta*-topolin has been etablished as an active aromatic cytokinin by Strnad et al. (1997). The sensitivity of the radish cotyledon bioassay to *m*T has not been established.

Peroxidase is a heme-containing enzyme associated with the regulation of indolacetic acid levels through oxidative catabolism (Barcelo and Munoz 1992). Peroxidase activity was measured as a morphogenic marker in cotyledons. Several of the developmental processes modulated by PA are also affected by cytokinins (Thimann 1980) and a pronounced effect of cytokinins on endogenous PA has been reported during the light-induced greening of etiolated cucumber cotyledons following incubation with BA (Suresh and Adiga 1977; Suresh et al. 1978). Polyamine levels in the radish cotyledons treated with *m*T were determined.

In order to assess the effects of *m*T on cellular metabolism and turnover of chlorophyll, total N and soluble protein contents were determined.

MATERIAL AND METHODS

Radish (*Raphanus sativus* L.) seeds were germinated in darkness for 4 d at 25°C on moist filter paper in 5-cm petri dishes. Cotyledons were excised excluding petiole tissues and four cotyledons were placed in each petri dishes after measuring the fresh weight. The cotyledons were placed with their adaxial sides down on the paper. They were incubated in a growth chamber at 25°C ± 2°C and 12 h light-dark photoperiods. Three millilitres of *m*T was applied per petri dish at 0.05, 0.1, 0.25, 0.5 and 1.0 mM concentrations. Cotyledon growth was measured by determining fresh and dry weights 3 d after the application (Letham 1971); the data presented are representative of 15 experiments. For chlorophyll determination, leaf segments were homogenized in 80% acetone. The samples were centrifuged

Abbreviations: **BA,** benzyladenine; ***m*T**, *meta*-topolin; **PA**, polyamine; **POD**, peroxidase

at 1000 × *g* for 5 min and the optical density of the supernatant was read at 663 and 645 nm with a spectrophotometer according to Arnon (1949); the data presented in Fig. 1A are representative of five experiments, with each treatment replicated four times. Peroxidase activity was analyzed in a reaction volume of 3 mL containing 0.1 M K-phosphate buffer, pH 5.8, 15 mM guaiacol and 5 mM H_2O_2. The reaction was started by the addition of an appropriate volume of the crude homogenate and the formation of tetraguaiacol was followed continuously for 2 min at 470 nm (Birecka et al. 1973). Data on POD activity presented are averages from seven experiments with each treatment replicated four times. The PA were extracted with 5% $HClO_4$ separated and detected after dansylation as described by Seiler and Wiechmann (1967). The data presented are average of four experiments with each treatment replicated four times.

The N content in the digested plant samples was determined by the procedure modified from Middleton (1960). Soluble protein content was determined as in Bradford (1976) using bovine serum albumin as standard. Each experiment was repeated four times and each treatment included three replicates.

RESULTS AND DISCUSSION

Meta-topolin in the 0.25 to 1 mM concentration range delayed the senescence in excised wheat leaf segments (Palavan et al., unpublished results). This concentration range was too high for radish cotyledon growth in preliminary experiments; therefore, we examined lower concentrations (0.05 to 1 mM) in the present study.

Cotyledon fresh weight accumulation increased at every increase in *m*T concentration, but dry weight accumulation of cotyledons increased only at the high rates of *m*T application (Table 1). Cytokinin treatment promotes cell expansion with no increase in the dry weight of the treated cotyledons (Huff and Ross 1975). Letham (1971) reported that cytokinins can promote markedly the expansion of radish cotyledons and attributed this response to the promotion of cell enlargement. *Meta*-topolin is an active aromatic cytokinin (Strnad et al. 1997) and stimulated cotyledon growth markedly.

Application of cytokinins promotes photosynthetic activity mainly by increasing of chlorophyll content, accelerating the conversion of etioplasts into chloroplasts, or modifying other components of photosynthesis, such as CO_2 assimilation capacity and activity of photosynthetic enzymes (Parthier 1979; Fletcher et al. 1982; Caers and Vendrig 1986). *Meta*-topolin increased the chlorophyll content over the control level at all applied concentrations (Fig. 1A). At 0.1 mM *m*T, the chlorophyll level was 35% greater than in the control cotyledons.

Meta-topolin increased the soluble protein contents of radish cotyledons. 0.1 and 0.25 mM *m*T treatments caused an increase in soluble protein levels of 4 and 6%, respectively, relative to the control cotyledons (Fig. 1B). There is a good evidence that cytokinins play a role in regulating protein synthesis (Tepfer and Fosket 1978).

An enhancement in total N content was observed with the application of *m*T (Fig. 1C). These changes largely reflect synthesis of proteins, because most of the N in any plant part is in protein. Therefore, the findings obtained in this study were closely associated with soluble protein contents.

Table 1. The effect of *meta*-topolin on dry weight and cotyledon growth of radish cotyledons

Treatment	Dry weight mg cotyledon^{-1}	Growth mg FW cotyledon^{-1}
Control	2.85 ± 0.08	6.72 ± 0.24
0.05 mM	3.08 ±0.14	8.32 ± 0.71*
0.1 mM	3.14 ± 0.16	10.47 ± 0.49*
0.25 mM	3.26 ± 0.24	11.61 ± 0.27*
0.5 mM	3.45 ± 0.06*	12.68 ± 1.38*
1.0 mM	3.57 ± 0.25*	15.68 ± 0.57*

*Significantly different from control values at $P < 0.05$.

Peroxidase activities were reduced by the application of *m*T. The inhibition ratios were between 37 and 66% at 0.05 to 1 mM *m*T treated cotyledons compared with control cotyledons (Fig. 2). There are two main mechanisms whereby POD can effect the differentiation of plant cells. Peroxidase activity can alter the concentration of phytohormones through ezymatic metabolism (Trewavas 1991). Peroxidase activity can catalyze cross-linking reactions in the plant cell wall and, therefore, cell expansion (Fry 1986). Thus, POD activity may regulate the endogenous levels of the main growth promoting factor indolacetic acid and the later stages of cellular growth resulting from the biophysical process of cell expansion in radish cotyledons.

Cytokinins have been reported to increase polyamine content in many plant systems including lettuce cotyledons (Cho 1983) and cucumber cotyledons (Suresh et al. 1978; Walker et al. 1988) and to stimulate arginine decarboxylase activity (Suresh et al. 1978; Mukhopodhyay et al. 1983; Palavan et al. 1984).

Polyamines have been implicated in cell growth and development of plants. The putrescine content in cucumber cotyledons is increased markedly by cytokinin application (Walker et al. 1988). In the present study, putrescine and spermine contents were not affected by *m*T application (Table 2). However, spermidine content decreased with increasing *m*T concentration.

The natural aromatic cytokinin *m*T is active in moderating radish cotyledon growth. The application of *m*T resulted in changes to fundamental parameters including chlorophyll content, protein and N content, POD activity and polyamine content, typical of cytokinin activity. The study illustrates the usefulness of radish assay in detecting *m*T.

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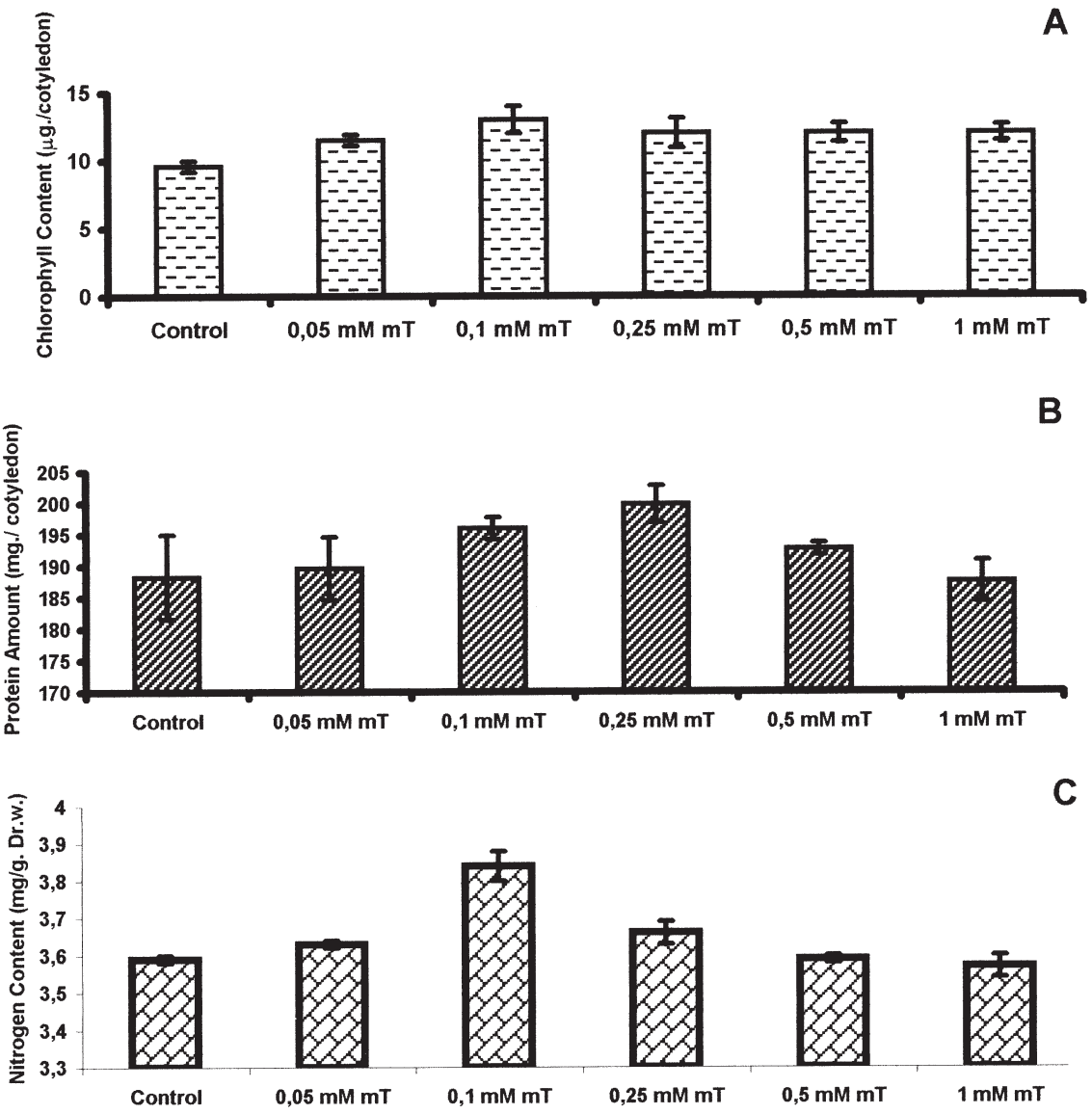

Fig. 1. The effect of *meta*-topolin on chlorophyll, soluble protein and N contents during the growth of radish cotyledons. Vertical bars represent standard errors. Values are average of four experiments.

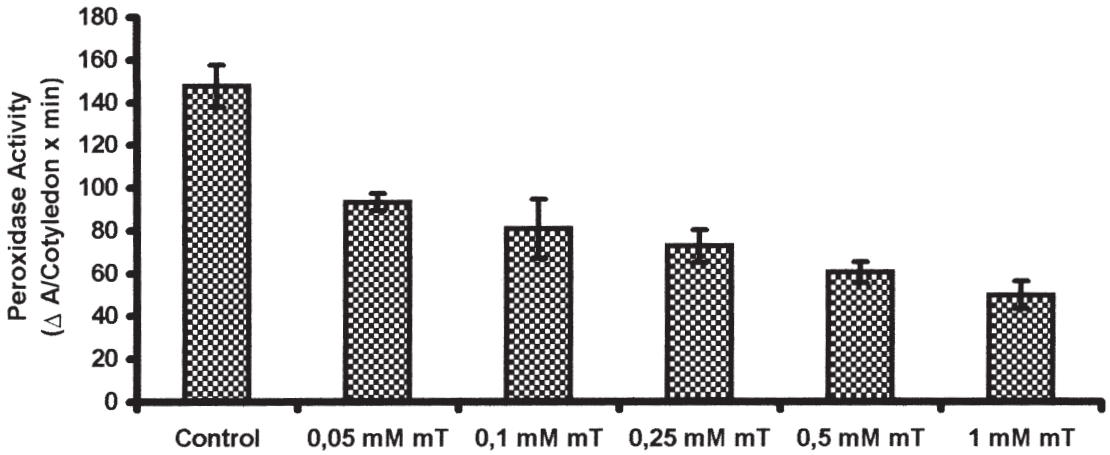

Fig. 2. The effect of *meta*-topolin on peroxidase activity during the growth of radish cotyledons. Vertical bars represent standard errors. Values are average of seven experiments.

Table 2. The effect of *meta*-topolin on polyamine contents during the growth of radish cotyledons. Values are average of four replicated experiments

Treatment	Polyamine content (ng cotyledone^{-1})		
	Spermine	Spermidine	Putrescine
Control	70.16 ± 18.33	585.23 ± 82.99	10307.00 ± 2739.54
0.05 mM	67.17 ± 16.92	445.75 ± 108.33	10835.00 ± 3643.63
0.1 mM	56.79 ± 15.42	415.20 ± 50.20	9124.41 ± 1915.80
0.25 mM	73.13 ± 39.8	605.03 ± 145.00	10167.61 ± 1497.51
0.5 mM	92.13 ± 7.25	306.62 ± 39.94*	6567.19 ± 1209.73
1.0 mM	109.83 ± 22.36	260.26 ± 37.00*	7410.21 ± 1737.01

*Significantly different from control values at $P < 0.05$.

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