Journal of Cell and Molecular Biology **2**: 31-34, 2003.
Haliç University, Printed in Turkey.

The effect of *meta*-topolin on protein profile in radish cotyledons

Serap Çağ[1] and Narçin Palavan-Ünsal[2]*
[1]*Istanbul University, Department of Biology, Botany Section, Süleymaniye 34460, Istanbul-Turkey;*
[2]*Haliç University; Department of Molecular Biology and Genetics, Fındıkzade 34280, Istanbul-Turkey*
(author for correspondance)*

Received 27 September 2002; Accepted 30 November 2002

Abstract

Meta-topolin (*m*T) has been established as an active aromatic cytokinin recently. The present investigation assessed the effects of *m*T on radish cotyledon growth and protein content. 0.05 to 1 mM *m*T increased the cotyledon growth about 2 fold in fresh weight basis. *m*T at 0.1, 0.25 and 0.5 mM concentrations caused an increase in soluble protein levels compared to the control cotyledons almost in the same ratio by 3 %. Compared to control cotyledons analysis of the soluble proteins displayed different electrophoretic pattern in *m*T treated cotyledons.

Key words: Cotyledon growth, *meta*-topolin, protein

Meta-topolinin turp kotiledonlarında protein profiline etkisi

Özet

Son yıllarda *meta*-topolin (*m*T) aktif aromatik sitokinin olarak saptandı. Bu araştırma da *m*T'in turp kotiledonlarının büyüme ve protein içeriğine etkisi araştırıldı. 0.05-1 mM *m*T kotiledon büyümesini taze ağırlık bazında yaklaşık 2 kat kadar teşvik etti. 0.05, 0.1 ve 0.25 mM *m*T çözünür protein düzeylerini kontrole oranla yaklaşık % 3 oranında arttırdı. Çözünür proteinlerin analizleri, *m*T uygulanan kotiledonlarda kontrole oranla farklı bir elektroforetik dizilim gösterdi.

Anahtar sözcükler: Kotiledon büyümesi, *meta*-topolin, protein

Introduction

Cytokinins, N^6-substituted adenine derivatives are a class of plant hormones that were first identified as factors that promoted cell division (Miller et al., 1955; 1956) and have been implicated in many other aspects of plant growth and development including shoot initiation and growth, apical dominance, senescence and photomorphogenetic development (Letham, 1971; Thimann, 1980; Mok and Mok, 1994). Although the physiological effects of cytokinins have been well documented, the molecular mechanisms underlying cytokinin action remain obscure (Mok and Mok, 1994; Binns, 1994).

Bioassays are used to establish the relative biological activity of plant hormones compared with others. The cytokinin bioassays used most frequently depend on growth of tissues in sterile culture (Letham 1967). Such methods are extremely sensitive but it needs at least 3 weeks to get final results. 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.

To date the effects of common cytokinins i.e. kinetin, benzyladenine (BA) and its riboside have been

documented in radish cotyledons. A new active aromatic cytokinin *meta*-topolin (*m*T) have been determined by Strnad et al. (1997) in poplar. The sensitivity of the radish cotyledon bioassay to *m*T has been established by us before (Palavan-Ünsal et al., 2002). This study will focus on the effect of *m*T on soluble protein contents in radish cotyledons that has not been studied before.

Material and methods

Plant material and bioassay

Radish (*Raphanus sativus* L.) seeds were germinated in darkness for 4 days 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 dish 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 ml *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 days after the application (Letham, 1971) and the data presented here representative of 15 experiments.

Measurement of soluble protein content

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.

Electrophoresis for proteins

Sodium dodecylsulphate (SDS)-polyacrylamide slab gel electrophoresis was performed according to Laemmli (1970). Gel containing 3.0 % (stacking gel) and 10.0 % (separation gel) acrylamide were prepared from a stock solution of 30.0 % of acrylamide and 0.8 % N, N'-bis methylene acrylamide. The gels were polymerized chemically by the addition of ammonium persulphate. The mixture was completely dissociated by immersing the samples for 3 min in boiling water. Electrophoresis was carried out with a current of 150 V per gel until the bromophenol blue marker reached the bottom of the gel. The proteins were stained in the gel with Coomassie brilliant blue solution for overnight at room temperature. The gels were diffusion-destained by repeated washing in the solution containing 7.5 % acetic acide, 5 % methanole and 87.5 % distilled water.

Results and discussion

The early observations revealed that cytokinins exert parallel effects in maintain protein or nucleic acid levels while inhibiting senescence. Cytokinins stimulate both structural and enzymatic protein synthesis. They are selectively increasing the levels of certain enzymes associated generally with photosynthetic process (Feierabend, 1969). It is not clear whether the enhanced activity is due to greater synthesis, inhibition of degradation or activation of the enzymes.

We already observed that new aromatic cytokinin *m*T at 0.25 to 1 mM concentration range delayed the senescence in excised wheat leaf segments (Palavan et al., 2002). This concentration range was high for radish cotyledon growth therefore lower concentrations were examined (0.05 to 1 mM) in addition.

Cotyledon growth increased with the treatments of *m*T significantly (Figure 1). Stimulation of cotyledon growth was closely related with increasing concentrations of *m*T; 0.05 to 1 mM *m*T increased the cotyledon growth about two fold in fresh weight basis ($p<0.05$), while dry weights of cotyledons during the growth were not effected by *m*T application (Palavan-Ünsal et al., 2002).

Cytokinins promote cell enlargement in certain tissues and organs. This effect is most clearly seen in cotyledons. The expansion of cotyledons is resulted

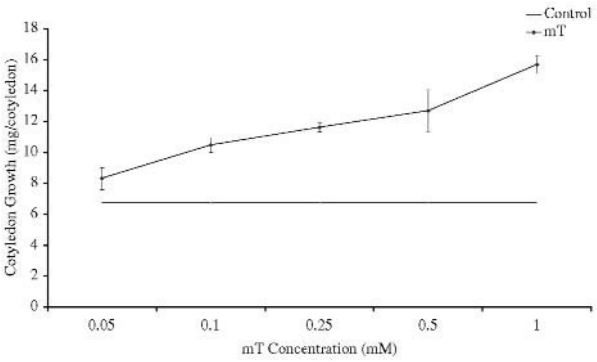

Figure 1: The effect of *meta*-topolin on cotyledon growth in radish (Values are average of 30 cotyledons).

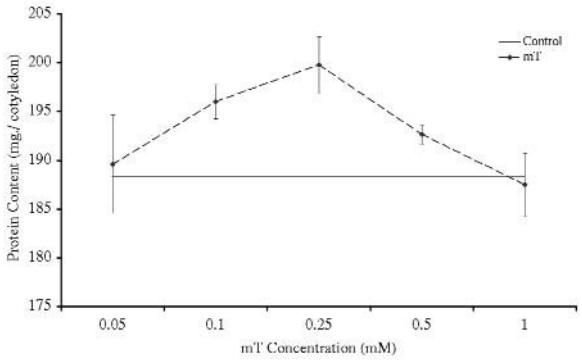

Figure 2: The effect of *meta*-topolin on soluble protein content during the growth of radish cotyledons. Values are average of 4 experiments.

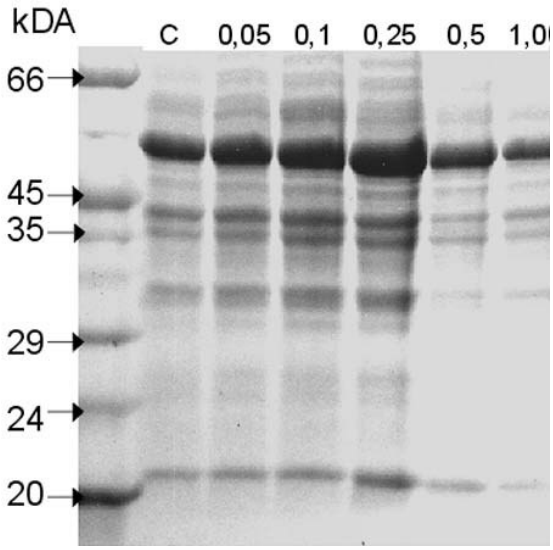

Figure 3: SDS-PAGE analysis of soluble proteins from *meta*-topolin treated radish cotyledons. Gel was stained with Coomassie blue. Lane 1: Control, Lane 2: 0.05 mM *m*T, Lane 3: 0.1 mM *m*T, Lane 4: 0.25 mM *m*T, Lane 5: 0.5 mM *m*T, Lane 6: 1 mM *m*T treatments. Molecular mass (kDa) of markers are indicated on left hand margin.

from cell enlargement during cotyledon growth. Cytokinin treatment promotes additional cell expansion with no increase in the dry weight of the treated cotyledons (Huff and Ross, 1975).

Letham (1971) reported the ability of cytokinins to promote markedly the expansion of radish cotyledons and explained this response by the promotion of cell enlargement. *m*T also as a most active aromatic cytokinin as reported by Strnad et al. (1997) caused to cotyledon growth markedly as shown in Figure 1.

*m*T was found to increase the soluble protein contents of radish cotyledons. Treatments with 0.1, 0.25 and 0.5 mM *m*T resulted an increase in soluble protein content in the same ratio (by 3,4 and 3 % respectively) compared to the control cotyledons (Figure 2).

These findings correlated with electrophoretic determinations (Figure 3). Soluble proteins of *m*T treated radish cotyledons were analyzed using SDS-PAGE technique in order to test whether and significant amount of difference in protein profile occurred with *m*T treatments. Analysis of the soluble proteins displayed different electrophoretic pattern in *m*T treated cotyledons compared to control. Protein bands were very sharp and dark in 0.05, 0.1 and 0.25 mM *m*T treated samples and their molecular masses ranges between 66 to 45 kDa's. Molecular mass of 45 to 29 kDa's were weak in 0.5 and 1 mM *m*T treated and in control cotyledons also. On the other hand protein bands were very sharp and dark in 0.05, 0.1 and 0.25 mM *m*T treated cotyledons comparing with 0.5 and 1 mM *m*T treated and control cotyledons. Obvious bands were also observed around 30 kDa in cotyledons treated with 0.05, 0.1 and 0.25 mM *m*T. Besides these there were additional bands in *m*T treated samples different from controls and these bands were weak in 0.5 and 1.0 mM *m*T treated samples compared to the other applications around 24 kDa.

There is good evidence that cytokinins play a role in regulating protein synthesis (Tepfer and Fosket, 1978). Cytokinins can not only increase the rate of protein synthesis, but also change the spectrum of proteins produced by plant tissues.

Results obtained in this study showed that, total soluble protein content in radish cotyledons not effected from exogenously applied *m*T. On the other hand, when protein profile was examined electrophoretically additional bands were observed in *m*T treated samples. These can be explained by the fact that *m*T stimulate new protein synthesis without effecting total protein content.

In conclusion, natural aromatic cytokinin *m*T has an important role in the control of cotyledon growth and this response closely associated with protein profile. The results of this research are exhibited *m*T as a promising plant growth regulators in physiological studies.

Acknowledgement

We thank to Dr. M. Strnad and his colleagues for the generous gift of aromatic cytokinins and to Damla Büyüktunçer for technical assistance. This study was supported by Istanbul University Research Fund (Project number: B-430/13042000).

References

Binns AN. Cytokinin accumulation and action: biochemical, genetic and molecular approaches. *Ann Rev Plant Physiol Plant Mol Biol.* 45: 173-196,1994.

Bradford AM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 72: 248-254, 1976.

Feierabend J. Der Einfluss von Cytokinin auf die Bildung von Photosyntheseenzyme im Roggenkeimlingen. *Planta.* 84: 11-29, 1969.

Huff AK, Ross CW. Promotion of radish cotyledon enlargement and reducing sugar content by zeatin and red light. *Plant Physiol.* 56: 429-433, 1975.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227: 680-685, 1970.

Letham DS. Chemistry and physiology of kinetin-like compounds. *Ann Rev Plant Physiol.* 18: 349-364, 1967.

Letham DS. Regulators of cell division in plant tissues. XII. A cytokinin bioassay using excised radish cotyledons. *Physiol Plant.* 25: 391-396, 1971.

Miller CO, Skoog F, Von Saltza, MH, Strong F. Kinetin a cell division factor from deoxyribonucleic acid. *J Am Chem Soc.* 77: 1392-1293, 1955.

Miller CO, Skoog F, Okomura FS, von Saltza MH, Strong FM. Isolation, structure and synthesis of kinetin a substance promoting cell division. *J Am Chem Soc.* 78: 1345-1350, 1956.

Mok DWS, Mok MC. *Cytokinins: Chemistry, Activity and Function.* CRC Press, Boca Raton. 1994.

Palavan-Ünsal N, Çağ S, Çetin E. Growth responses of excised radish cotyledons to *meta*-topolin. *Canadian J Plant Sci.* 82: 191-194, 2002.

Strnad M, Hanus J, Vanek T, Kaminek M, Ballantine JA, Fussell B, Hanke DE. *Meta*-topolin, a highly active aromatic cytokinin from poplar leaves (*Populus x canadensis* Moench., cv. *Robusta*). *Phytochemistry.* 45: 213-218, 1997.

Tepfer DA, Fosket DE. Hormone-mediated translational control of protein synthesis in cultured cells of *Glycine max. Dev Biol.* 62: 486-497, 1978.

Thimann KV. *Senescence in Plants.* 85-115. CRC Press, Boca Raton. 1980.