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BRIEF COMMUNICATION

Effect of ethylene and its antagonist 1-MCP on the senescence of detached leaves of *Arabidopsis thaliana*

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Abstract

1-Methylcyclopropene (1-MCP) applied alone did not influence significantly the chlorophyll and carotenoid content of the older leaves of *Arabidopsis thaliana* (L.) Heynh., but retarded the senescence of the younger ones (6th and 7th leaf nodes). However, 1-MCP effectively blocks the ethylene induced senescence of excised rosette leaves. The preliminary application of 1-MCP (3 h in advance to the treatment by *Ethrel*) almost totally eliminated the ethylene action. Similar trend was also observed after simultaneous application of *Ethrel* and 1-MCP, and the effects of both treatments on the chlorophyll and carotenoid destruction are comparable.

Additional key words: carotenoids, chlorophyll, ethylene releasing agent, leaf node.

The leaf yellowing is one of the first visible symptoms of senescence – natural or induced by various unfavourable environmental factors (Thomas and Stoddart 1980, Zacarias and Reid 1990). It is well known that the ethylene production is enhanced during the leaf ageing (Nichols 1966) and this process can be accelerated by the application of ethylene (Matoo and Aharoni 1988, Zacarias and Reid 1990). On the other hand there are number of compounds which can diminish the ethylene effects – cytokinins, Ag-thiosulphate, CO_2, nitric oxide, nitrous oxide, 2,5-norbornadiene, cyclooctene, cyclo-octadiene, some derivatives (esters and hydrazides) of the dicarboxylic acids, *etc.* (Sisler and Pian 1972, Alexieva 1987, Goldthwaite 1988, Sisler *et al*. 1990, 1996, Leshem and Wills 1998). Recently, 1-methylcyclo-propene (1-MCP) is a wide used as one of the strongest ethylene antagonist (Sisler *et al*. 1996, Zhong *et al*. 2001).

The aim of this study was to establish the effectiveness of 1-MCP in preventing the senescence of detached leaves of wild type *Arabidopsis thaliana* caused by the ethylene releasing agent *Ethrel* (2-chloroethyl-phosphonic acid).

A wild type of *Arabidopsis thaliana* (L.) Heynh, cv. Columbia were used. The plants were grown in plastic pots (d = 70 mm, h = 80 mm), filled with soil/*Perlite* mixture (3:1) in a growth chamber (16-d photoperiod, 70 μmol m^{-2} s^{-1} photon flux density, 24/20 °C day/night temperature, 60 % air humidity). The plants were daily irrigated. Rosette leaves (3rd to 7th leaf node) from 30-d-old plants were cut, weighed and placed in Petri dishes (d = 100 mm) for incubation on two layers of filter paper wetted with 5 cm^3 of distilled water (control) or *Ethrel* (0.001 cm^3 dm^{-3}). The 1-MCP treatment was performed for 3 h (prior to, or along with the application of *Ethrel*) by injection (0.00012 cm^3 dm^{-3}) in a sealed glass chambers containing a set of excised leaves in Petri dishes. After that all samples were incubated in darkness (25 °C) for 48 h and chlorophyll and carotenoid content was measured spectrophoto-metrically (Arnon 1949). 1-MCP was synthesized

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Abbreviations: Car - carotenoids; Chl - chlorophyll; 1-MCP - 1-methylcyclopropene
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Effect of temperature stress on the endogenous cytokinin content in *Arabidopsis thaliana* (L.) Heynh plants

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Key words: *Arabidopsis thaliana* (L.) Heynh, cytokinins, ELISA, ethylene-insensitive mutant *(eti5),* temperature stress

Abstract

The levels of three endogenous cytokinin equivalents: zeatin (Z), *iso*-pentenyladenine (iP) and dihydrozeatin (dZ) in two *Arabidopsis thaliana* (L.) Heynh genotypes – wild type (wt) and ethylene-insensitive mutant (*eti5*), were compared using enzyme immunoassay (ELISA). Cytokinin content was measured after exposure to low (4 °C for 24 h in darkness) or high temperature (38 °C for 24 h in darkness). Measurements were performed immediately and 24, 48 and 120 h after treatments. It was found that at normal growth conditions *eti5* plants contained more endogenous cytokinins compared to the wild type. At both temperature treatments mutant plants had decreased total cytokinin levels. Wild-type plants treated with high temperature (HT) exhibited reduced total cytokinins (with the exception of rates at 48 h), while low temperature (LT) treatment resulted in elevated total amount of the studied equivalents (except at 24 h). The obtained results suggested that HT had greater effect on cytokinin levels than LT since it caused more profound changes in the total content. We assume that this was due to the natural chilling tolerance of *Arabidopsis* plants.

List of abbreviations: dZ – dihydrozeatn, dZR – dihydrozeatin riboside, ELISA – enzyme linked immunosorbent assay, HT – high temperatute, iP – *iso*-pentenyladenine, iPA – *iso*-pentenyladenosine, LT – low temperature, SE – standard error, wt – wild type, Z – zeatin, ZR – zeatin riboside

Introduction

Senescence as programmed ageing process leads to plant death (Dangl *et al.* 2000). According to Nooden *et al.* (1997) leaf senescence resembles processes occurring at oxidative stress. Levels of reactive oxygen species increased during senescence likewise after environmental stress (Merzlyak and Hendry 1994). The plant responses to different environmental stresses are specifically mediated by plant hormones. Cytokinins play an important role in several aspects of plant growth, metabolism and development at normal growth conditions. The mechanisms by which environmental changes affect cytokinins are still not clear but their adaptive function is undoubted. It is suggested that cytokinins

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Antagonistic effects of triazolo[4,5-d]pyrimidine and pyridylurea derivatives on cytokinin-induced cytokinin oxidase/dehydrogenase activity in young pea plants

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Key words: CPPU, Cytokinin antagonists, Cytokinin oxidase/dehydrogenase activity, N^6-benzyladenine

Abstract

The effect of strong and weak cytokinin antagonists, belonging to the groups of triazolo[4,5-d]pyrimidines (TP), and pyridyl-phenylurea derivatives (PU), on cytokinin oxidase/dehydrogenase activity (CKX) in the tissues of young pea plants was studied. Tested anticytokinins, with the exception of the most efficient one – PU-1, were able to promote increased CKX activity in roots, when applied alone, but they had no significant influence on the enzymatic activity in leaves. N^6-benzyladenine (BA) and 1-(2-chloropyridin-4-yl)-3-phenylurea (CPPU) provoked strong increase in CKX activity in roots, while in leaves considerable inhibition of enzymatic activity was observed. Different types of anticytokinins exhibited diverse preference towards taking off the action of purine and phenylurea cytokinins over CKX activity.

Abbreviations: BA – N^6-benzyladenine; CKX – cytokinin oxidase/dehydrogenase activity; CPPU – 1-(2-chloropyridin-4-yl)-3-phenylurea; PU-1 – 3-[(pyridin-2-yl)methyl]-1-phenylurea; PU-9 – 3-[(pyridin-4-yl) methyl]-1-phenylurea); TP-7 – *tert*.butyl 2-(3-benzyl-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7-ylamino)acetate; TP-8 – 2-(3-benzyl-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7-ylamino)acetohydrazide

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\Cytokinin oxidase/dehydrogenase (CKX) activity in wild and ethylene-insensitive mutant *eti5* type of *Arabidopsis thaliana* (L.) Heynh plants and the effect of cytokinin N^1-(2-chloro-4-pyridyl)-N^2-phenylurea on enzymatic activity and leaf morphology

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Key words: *Arabidopsis thaliana* (L.) Heynh, cytokinin oxidase/dehydrogenase, cytokinin 4PU-30, ethylene-insensitive (*eti5*) mutant.

Abstract

The specific activity of cytokinin oxidase/dehydrogenase (EC 1.5.99.12) (CKX) was determined in leaves of wild type (*wt*) and ethylene-insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh plants. Comparative studies showed that this mutation has lower basal CKX activity than *wt*. Application of 4PU-30 (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea) resulted in decreased CKX activity in both *wt* and mutant plants. The treatment increased leaf blade thickness and the volume of chlorophyll-containing cells per unit leaf area in *wt* but these changes were not observed in the *eti5* mutant. The reduction in chlorophyll "a" and "b", as well as in carotenoids content in the treated *wt* tissues resulting from altered leaf morphology was not detected in *eti5* plants.

List of abbreviations: 4PU-30 - N^1-(2-chloro-4-pyridyl)-N^2-phenylurea, CKX - cytokinin oxidase/dehydrogenase; iP – *iso*-pentenyladenine; SE - Standard error; FW - fresh weight; dZR - dihydrozeatin riboside, iPA – *iso*-pentenyladenosine, *wt*- wild type, ZR - zeatin riboside; dZ – dihydrozeatin; Z – zeatin.

Introduction

Transgenic and hormone mutant plants are convenient model systems for the investigation of various physiological processes. Earlier, it has been established that the ethylene-insensitive mutant *eti5* of *Arabidopsis* has characteristics of delayed senescence accompanied by enhanced ethylene biosynthesis (Harpham *et al.* 1991), a higher amount of leaf pigments and soluble proteins (Sergiev *et al.* 2003), as well as elevated endogenous polyamines (Todorova *et al.* 2002) and cytokinins (Kudryakova *et al.* 2001, Todorova *et al.* 2005) than the wild type. *Eti5* mutation is a very curious case of co-existence of two factors, which counteract each other in relation to senescence – firstly higher ethylene levels promoting it and secondly higher cytokinin content that delays the process. The abnormally elevated cytokinin levels in *eti5*

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BRIEF COMMUNICATION

Influence of cytokinins and novel cytokinin antagonists on the senescence of detached leaves of *Arabidopsis thaliana*

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Abstract

Cytokinins N^6-benzyladenine (BA) and 1-(2-chloropyridin-4-yl)-3-phenylurea (4PU-30) delayed the senescence of detached leaves (3rd to 7th leaf node) of wild and ethylene insensitive *eti5* mutant of *Arabidopsis thaliana*. The novel anticytokinins, structural analogues of purine and phenylurea cytokinins also affected the senescence of detached rosette leaves of *A. thaliana*. They diminished to a significant extent the cytokinin-induced delay of chlorophyll destruction, but without a considerable difference in their action against both types of cytokinins. These results correlated with changes observed in ribonuclease (RNase) activity.

Additional key words: anticytokinins, chlorophyll, ethylene insensitive mutant, leaf node, RNase.

Senescence (natural or induced by various artificial factors) is accompanied by destruction of photosynthetic pigments and leaf yellowing is one of the first visible symptoms of this process (Thomas and Stoddart 1980, Zacarias and Reid 1990, Sergiev *et al*. 2003, Todorov *et al*. 2003a,b, Alexieva *et al*. 2004). The leaf isolation is a key event leading to commencement of the senescence mechanisms. The growth promoting as well as senescence-delaying properties of cytokinins and their action as anti-senescence agents are wide studied (Van Staden *et al*. 1988, Zacarias and Reid 1990, Stoynova-Bakalova *et al.* 2001, Wilhelmová *et al.* 2004). On the other side, the application of cytokinin antagonists eliminates the cytokinin-induced hindrance of senescence in excised leaves and other model systems (Karanov *et al*. 1993, Alexieva *et al*. 1994, Sergiev 1999). The destruction of RNA and induction of RNase activity are characteristic for the senescence in higher plants (Dangl *et al*. 2000). To our knowledge, the effects of cytokinin antagonists in relation to senescence-induced changes in RNase activity are not studied.

Arabidopsis mutants have been used increasingly in physiological and biochemical studies (Kieber 1997). We used plants of *Arabidopsis thaliana* (L.) Heynh. wild type (WT), and the ethylene insensitive mutant *eti5*. This mutant (Harpham *et al*. 1991) possesses characteristics of delayed senescence accompanied with higher amount of leaf pigments and soluble proteins (Sergiev *et al*. 2003, Todorov *et al*. 2003a,b). In the present work we investigated the effect of synthetic purine and phenylurea cytokinins and their novel structural analogues with anticytokinin properties on chlorophyll (Chl) content and RNase activity in detached rosette leaves.

The plants were grown in plastic pots, filled with soil/*Perlite* mixture (3:1) in a growth chamber (16-h photoperiod, 70 μmol m^{-2} s^{-1} photon flux density, 24/20 ºC day/night temperature, 60 % air humidity). The plants were daily irrigated. In order to characterize the senescence of detached rosette leaves in presence of purine and phenylurea cytokinins, their antagonists

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Abbreviations: BA - N^6-benzyladenine; 2PU-3 - 1-(4-chlorophenyl)-3-(pyridin-2ylmethyl)urea; 4PU-30 - 1-(2-chloropyridin-4-yl)-3-phenylurea; RNase - ribonuclease; TP-5 - 3-benzyl-7-(4-methylpiperazin-1-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine.
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BRIEF COMMUNICATION

Cytokinin oxidase/dehydrogenase activity as a tool in gibberellic acid/cytokinin cross talk

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Abstract

Changes in endogenous cytokinin (CK) content and cytokinin oxidase/dehydrogenase activity (CKX) in response to gibberellic acid (GA_3) in two pea cultivars with different life span were assessed. The control leaves of cv. Scinado, which developed faster, had higher initial cytokinin content and lower CKX activity, while opposite trend was observed in cv. Manuela with longer life span. Increased CKX and decreased CK content were detected in leaves of cv. Scinado after treatments with 0.5, 1 and 5 μM GA_3. Changes in CK content and CKX activity in GA_3-treated cv. Manuela leaves were reciprocal to those in cv. Scinado. CK content and CKX activity in roots were not significantly influenced by the application of GA_3. The slight repression of CKX activity in some of the root samples was accompanied by increased isopentenyladenine and isopentenyladenine riboside content. Obtained results suggest that CKX was responsible for the changes in endogenous cytokinin pool in GA_3-treated plants and most probably this enzyme represents an important link in GA/cytokinin cross talk.

Additional key words: isopentenyladenine, pea, *Pisum sativum*, zeatin.

Gibberellins (GAs) and cytokinins (CKs) control different developmental processes in plants. CKs act early during shoot initiation and control meristem activity, while GAs are responsible for expansion and cell division in shoot elongation, flowering and seed germination. All phytohormones exert their regulatory role in close relation with each other. Hormone signalling pathways form complex interacting network, which enables perceiving of numerous internal and external stimuli and generating respective plant responses. Additionally, exogenously applied growth regulators can alter the content of endogenous phytohormones (Pospíšilová 2003). Thus hormones may influence a wide range of physiological events during plant growth and development acting both in synergism and in antagonism with other phytohormones. Relatively little information is available concerning interaction between GAs and CKs. Both hormones delay senescence in some plant species (Jacob-Wilk *et al.* 1999, Mok and Mok 1994) and induce stomatal opening (Pospíšilová 2003). Huang *et al.* (2003) showed that GAs and CKs promote male development in tobacco and *Arabidopsis*. An antagonistic action of these two hormones was reported by Greenboim-Wainberg *et al.* (2005) in wild type *Arabidopsis* plants, where GA_3 and benzyladenine (BA) had opposite effect on anthocyanin accumulation and root elongation. Earlier

Received 10 April 2006, *accepted* 20 September 2006.
Abbreviations: ABA - abscisic acid; BSA - bovine serum albumin; *cis*Z - *cis* zeatin; *cis*ZR - *cis* zeatin riboside; *cis*ZRP - *cis* zeatin riboside monophosphate; CK - cytokinin; CKX - cytokinin oxidase/dehydrogenase; GA - gibberellin; GA_3 - gibberellic acid; HPLC - high performance liquid chromatography; iP - isopentenyladenine; iPR - isopentenyladenine riboside; iPRP - isopentenyladenine riboside monophosphate; PMSF - phenylmethylsulfonyl fluoride.
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ORIGINAL PAPER

Polyamine content in *Arabidopsis thaliana* (L.) Heynh during recovery after low and high temperature treatments

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Abstract Comparative studies on the effect of temperature treatment on the endogenous polyamine content in wild type and the ethylene insensitive mutant *eti5* of *Arabidopsis thaliana* (L.) Heynh were performed. The levels of free and conjugated putrescine, spermidine and spermine were measured in rosette leaves of 38-day-old plants subjected to low and high temperature for 24 h in darkness. Data for fractions measured in treated wild type plants during recovery suggest that alterations in polyamine levels may be a consequence of the conversion of the supernatant-bound into free form and *vice versa*, while in treated *eti5* plants *de novo* synthesis of spermidine and spermine could not be excluded. It was found that high temperature provoked more significant changes in polyamine levels than low temperature. The results suggest that the *eti5* mutant showed a better ability to recover after the temperature treatments than wild type partly as a consequence of changes in polyamine content.

Keywords *Arabidopsis thaliana* (L.) Heynh · Ethylene insensitive mutant (*eti5*) · High temperature · Low temperature · Polyamines · Putrescine · Spermidine · Spermine

Abbreviations

HT	High temperature (38°C)
LT	Low temperature (4°C)
PA	Polyamine
Put	Putrescine
Spd	Spermidine
Spm	Spermine
TCA	Trichloroacetic acid
Wt	Wild type

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Introduction

Polyamines (PAs) are organic molecules, widely distributed in both higher and lower plants. The PAs common for all plant species are putrescine (Put), spermidine (Spd) and spermine (Spm). They occur as free molecules, but can also be conjugated with small molecules such as phenolic acids or macromolecules like nucleic acids and proteins (Bouchereau et al. 1999). Like plant hormones, PAs may promote growth and provoke

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ORIGINAL PAPER

Response of cytokinin pool and cytokinin oxidase/dehydrogenase activity to abscisic acid exhibits organ specificity in peas

Irina Vaseva · Dessislava Todorova · Jiří Malbeck · Alena Trávníčková · Ivana Macháčková

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Abstract Changes in cytokinin pool and cytokinin oxidase/dehydrogenase activity (CKX EC: 1.5.99.12) in response to increasing abscisic acid (ABA) concentrations (0.5–10 μM) were assessed in the last fully expanded leaves and secondary roots of two pea (*Pisum sativum*) varieties with different vegetation periods. Certain organ diversity in CKX response to exogenous ABA was observed. Treatment provoked altered cytokinin pool in the aboveground parts of both studied cultivars. Specific CKX activity was influenced significantly basically in roots of the treated plants. Results suggest that ABA-mediated cytokinin pool changes are leaf-specific and involve certain root signals in which CKX activity presents an important link. This enzymatic activity most probably regulates vascular transport of active cytokinins from roots to shoots.

Keywords Abscisic acid · Cytokinins · Cytokinin oxidase/dehydrogenase · Organ-specific response · *Pisum sativum*

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Introduction

Abscisic acid (ABA) mediates environmental stress signals resulting from drought, high salinity, and low temperature, which is usually manifested at the physiological level by controlling germination, stomatal movements, and growth (Hoth et al. 2002). Balance between abscisic acid and cytokinins, was found to be one of the basic factors which control the stomatal aperture effectively (Das et al. 1976).

Significant cross talk occurs between various plant hormone signal transduction cascades initiating also the process of ageing. Ethylene and ABA normally induce the early onset of senescence. Generally endogenous levels of cytokinins (together with those of auxin and gibberellins) decline before and with the appearance of senescence (Van Staden et al. 1988).

Antagonistic relationship between cytokinin and ABA was demonstrated in an earlier study where application of abscisic acid inhibited the cytokinin-stimulated formation of buds in the moss *Funaria hygrometrica* (Valadon and Mummery 1971). The induction of shoot buds in the moss *Funaria hygrometrica* is a classic quantitative bioassay for cytokinins. Later Christianson (2000) localized the inhibitory action of ABA to a particular time in the process of bud formation. Experiments transferring protonema between cytokinin and cytokinin plus ABA show that ABA does not interfere with the initial perception of cytokinin. ABA acted later by blocking the cytokinin-mediated stable commitment of nascent buds—the so-called second cytokinin-mediated event in the process of bud formation. This event was also responsible for the concentration-dependent regulation of the overall number of buds that will be produced from individual protonemata (Christianson 1998). Obtained results revealed that initial perception of cytokinin and the second cytokinin-mediated

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ORIGINAL PAPER

Endogenous polyamines lessen membrane damages in pea plants provoked by enhanced ultraviolet-C radiation

Zornitsa I. Katerova · Dessislava Todorova

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Abstract The effect of low doses (LD)—0.1 kJ m^{-2} d^{-1} and high doses (HD)—0.3 kJ m^{-2} d^{-1} of UV-C irradiation on free, conjugated and bound spermine, spermidine and putrescine in leaves of young pea plants after 7 and 14 days of consecutive treatment was studied. Free polyamine (PA) fractions increased mainly in LD treated plants, while conjugated fractions decreased. Bound fractions accumulated mainly at the end of the experiment (after 14 days of UV-C irradiation). The results are interpreted in relation to the possible role of endogenous bound PAs in the prevention of membrane damage induced by UV-C irradiation. Stress markers (malondialdehyde and electrolyte leakage) increased after 7 days of UV-C treatment, and reached control values by the end of the experiment (mainly after HD treatment). Malondialdehyde concentration correlated negatively with UV-C—induced bound fraction and total PAs. The results support the conclusion that endogenous PAs lessen membrane damage in young pea plants provoked by UV-C irradiation.

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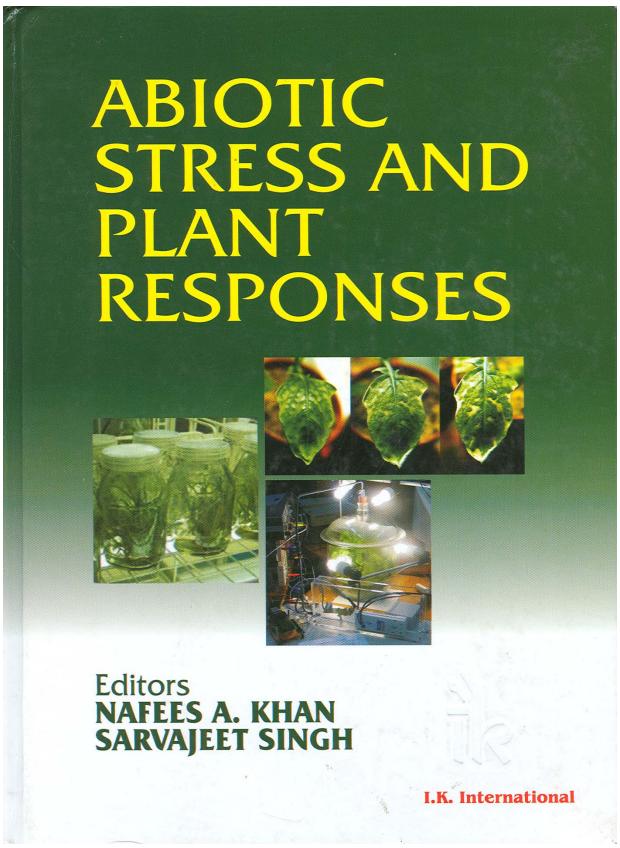
Keywords *Pisum sativum* · Polyamines · Putrescine · Spermidine · Spermine · Ultraviolet-C radiation

Abbreviations

DAO	Diamine oxidase
HD	High dose UV-C
MDA	Malondialdehyde
LD	Low dose UV-C
PA	Polyamine
PAO	Polyamine oxidase
Put	Putrescine
Spd	Spermidine
Spm	Spermine
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TLC	Thin layer chromatography

Introduction

The reduction in the ozone layer resulting from human activities has led to a significant increase in UV radiation which reaches the Earth's surface, which has deleterious effects on plant growth, development and productivity. Solar UV radiation has been classified as: UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm). The damaging effect of UV radiation increases towards shorter wavelengths: UV-A is less effective than UV-B with the exception of singlet



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11

Changes in Endogenous Polyamines and Some Stress Markers Content Induced by Drought, 4PU-30 and Abscisic Acid in Wheat Plants

D. Todorova[1], I. Moskova[1], I. Sergiev[1], V. Alexieva[1]* and S. Mapelli[2]

ABSTRACT

Aerial parts of 7-day old wheat (*Triticum aestivum* L.) seedlings were sprayed with a water solution of the phenylurea cytokinin 4PU-30 or ABA. Twenty-four hours later, part of them was subjected to moderate (– 0.2MPa) or strong (–1.0MPa) water stress. It was found that both stresses induced an accumulation of free, SN-bound and pellet-bound Put in shoots and roots of wheat plants. Free Spd and Spm fractions were little affected by drought in both organ tissues. SN-bound and pellet-bound fractions of Spd and Spm were enhanced in shoots, but in roots strong water deficit provoked a decrease in these fractions. The application of ABA and 4PU-30 caused a slight rise in polyamine levels. In combination with water deficit they increased almost all polyamine levels, but the effect on the free Put in shoots was most substantial. Water shortage also provoked membrane integrity deterioration, mainly due to the lipid peroxidation. Both plant growth regulators significantly reduced the malondialdehyde levels and free proline content of drought-treated wheat seedlings. The results obtained present additional information about the physiological role of growth regulators in relation to water stress.

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MILD TEMPERATURE STRESS MODULATES CYTOKININ CONTENT AND CYTOKININ OXIDASE/DEHYDROGENASE ACTIVITY IN YOUNG PEA PLANTS

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Cytokinin oxidase/dehydrogenase (CKX: EC 1.5.99.12) is able to provide a means for the rapid turnover of its substrate and it has been considered responsible for changes in the cytokinin pool in an adverse environment. Mild temperature stresses (10°C and 33°C average) were applied to young pea plants of two varieties (cvs. Manuela and Scinado) in order to assess the response of the cytokinin pool and CKX activity to altered growth conditions. Both temperature treatments increased the isopentenyl adenine (iP) and isopentenyl adenine riboside (iPR) contents in stressed plants. This trend was far more pronounced in the leaves. Low temperature additionally resulted in elevated *cis* zeatin riboside (*cis*ZR) and CKX activity. Heat did not influence the enzymatic activity in the leaves, while opposing trends were observed in the root-derived CKX activity of the two tested varieties. The data suggest that variance in the temperature provokes adaptive reactions in the cytokinin pool, which is maintained by CKX activity.

Key words: cytokinins, cytokinin oxidase/dehydrogenase, pea, temperature stress

Abbreviations: *cis*Z – *cis* zeatin, *cis*ZR – *cis* zeatin riboside, *cis*ZRP – *cis* zeatin riboside monophosphate, CK – cytokinins, CKX – cytokinin oxidase/dehydrogenase, iP – isopentenyl adenine, iPR – isopentenyl adenine riboside, iPRP – isopentenyl adenine riboside monophosphate.

Introduction

Hormonal changes are controlled by numerous signals and enzymatic pathways which govern plant acclimation to environmental stress (Mok and Mok, 2001). CK titres are usually modified by unfavourable growth conditions such as drought, water deprivation, excess salinity, changes in nutrient solutions, pathogen infection and wounding (Hare et al., 1997), high metal concentration (Atanasova et al., 2004) and herbicide treatment (Atanasova et al., 2005). Cytokinin changes, together with alterations in other endogenous plant growth regulators, may initiate physiological mechanisms involved in various protective plant stress responses.

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APPLICATION OF NATURAL AND SYNTHETIC POLYAMINES AS GROWTH REGULATORS TO IMPROVE THE FREEZING TOLERANCE OF WINTER WHEAT (*Triticum aestivum* L.)

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Wheat cultivars were grown as soil culture under normal growth conditions. Two-week-old seedlings were exposed to 4°C for 6 h and then transferred to –12°C for 24 h in the dark. Twenty-four hours before freezing stress, some of the plants were sprayed with aqueous solutions of spermine, spermidine, putrescine, 1,3-diaminopropane (1,3-DAP) and diethylenetriamine (DETA). The data showed that freezing stress caused a decrease in the fresh weight, chlorophyll content and plant survival rate, accompanied by a simultaneous accumulation of free proline and the enhanced leakage of electrolytes. Preliminary treatment with polyamines caused a decline in electrolyte leakage and a considerable augmentation in proline quantity, indicating that the compounds are capable of preventing frost injury. Additionally, the foliar application of polyamines retarded the destruction of chlorophyll, and lessened fresh weight losses due to freezing stress. The synthetic triamine DETA was the most effective, having the most pronounced action in all the experiments, followed by the tetraamine spermine. The application of polyamines to wheat crops could be a promising approach for improving plant growth under unfavourable growth conditions, including freezing temperatures. The results demonstrate that treatment with polyamines could protect winter wheat by reducing the stress injuries caused by subzero temperatures.

Key words: freezing temperature, stress, growth regulators, survival, electrolyte leakage

Introduction

Plants are able to acquire tolerance to various environmental stresses including cold. The development of cold tolerance involves many physiological, genetic and metabolic processes (Thomashow, 1990; Sãulescu and Braun, 2001; Yadav, 2010). Additionally, cold tolerance can be increased not only by cold hardening at low temperatures, but also by the preliminary application of plant growth regulating substances, such as abscisic acid (Gusta et al., 2005, and references therein), cytokinins (Jeon et al., 2010), salicylic acid, (Wang et al.,

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Ecophysiology and Responses of Plants under Salt Stress

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Chapter 13
Role of Polyamines in Alleviating Salt Stress

Dessislava Todorova, Zornitsa Katerova, Iskren Sergiev, and Vera Alexieva

13.1 Introduction

Abiotic and biotic stresses cause alterations in the normal physiological processes of all plants, including the economically important crops. Plant damage and decrease in their productivity take place most often due to naturally occurring unfavorable factors of the environment (natural stress factors). These include extreme temperatures; water deficit or abundance; increased soil salinity; high solar irradiance; early autumn or late spring ground frosts; pathogens, etc. Along with these factors, plants are imposed to a large scale of new stressors related to human activity (anthropogenic stress factors) including, toxic pollutants such as pesticides, noxious gasses (SO_2, NO, NO_2, NO_x, O_3 and photochemical smog); photooxidants; soil acidification and mineral deficit due to acid rains; overdoses of fertilizers; heavy metals; intensified UV-B irradiation, etc. (Fig. 13.1). All these stresses cause an increased production of reactive oxygen species (ROS) in plants that alter their normal physiological functions, decrease the biosynthetic capacity of plant organisms, and cause damages which may lead to plant death (Mittler 2002; Ahmad et al. 2008; Gill and Tuteja 2010b; Potters et al. 2010).

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POLYAMINE LEVELS IN *ARABIDOPSIS THALIANA* (L.) HEYNH, PLANTS DURING THEIR DEVELOPMENT[1]

D. Todorova, V. Alexieva, E. Karanov

(*Submitted on December 20, 2001*)

Abstract

Comparative studies were performed on the endogenous polyamines content in the wild type and in an ethylene insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh. We studied the level of free and bound putrescine (Put), spermidine (Spd) and spermine (Spm) in rosette leaves of plants during different stages of plant development. It was established that mutant plants contained a higher level of endogenous polyamines comparing to the wild type. In general, the highest levels of polyamines were detected in trichloracetic acid (TCA)-insoluble fraction and the lowest – in supernatant-bound (SN-bound) polyamines (PAs) in both genotypes. The results indicated a specific correlation between the high level of total spermidine and flower formation and seed germination, too. Not so significant increase was detected in total putrescine and spermine amounts during generative phases comparing to the vegetative phase. We suggested that spermidine plays the most important role in flowering and seed maturation.

Key words: *Arabidopsis thaliana* (L.) Heynh, ethylene insensitive mutant (*eti5*), flowering, maturation, polyamines, putrescine, spermidine, spermine

Introduction. PAs are organic molecules which are widely distributed in both higher and lower plants. PAs common for all plant species are Put, Spd and Spm [1]. PAs have specific features, for example, interference with senescence [2] and biosynthesis of the senescence hormone ethylene [3]. Like some phytohormones, PAs can induce growth and can provoke developmental changes and may be considered as a new class of endogenous growth regulators [4].

It is known that PAs play an important role in cell cycle, division and differentiation, flowers formation, fruits set and development, seeds germination. Their functions in these physiological processes in a lot of plant species are well documented [5–10]. However, in our knowledge, any information about endogenous polyamine levels in *Arabidopsis thaliana* plants during their development has not been published yet.

The aim of our work was to study the polyamine levels in two genotypes of *Arabidopsis thaliana* (*L.*) Heynh, plants during three different phases of plant development.

Materials and methods. PLANT MATERIAL AND GROWTH CONDITIONS. Wild type and ethylene insensitive mutant (*eti5*) plants of *Arabidopsis thaliana* (L.) Heynh, were grown in a growth chamber on soil/perlite mixture (3:1) under normal conditions (16/8 h day/night photoperiod; 70 μmol.m^{-2}.s^{-1} photon flux density; 26/22 °C

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BIOLOGIE

Physiologie des plantes

ENDOGENOUS FREE AND BOUND POLYAMINE CONTENT IN TOBACCO PLANTS SUBJECTED TO HIGH TEMPERATURE STRESS

D. Todorova, D. Parvanova*, T. Konstantinova*, S. Ivanov, D. Djilianov*, V. Alexieva

(*Submitted by Corresponding Member E. Karanov on March 26, 2003*)

Abstract

The levels of free and bound putrescine, spermidine and spermine were measured in leaves of tobacco plants subjected to high temperature treatment for 7 days. Polyamine contents were determined at 24, 74 and 168 h after the beginning of the stress programme. It was established that moderate enduring high temperature stress provoked a rise of the free putrescine as a stress marker, as well as bound to macromolecules polyamines which play a protective role against the damaging oxygen species. However, continuous stress led to a decrease of polyamine levels and diminished possibility for plant survival.

Key words: high temperature stress, *Nicotiana tabacum* L., polyamine, putrescine, spermidine, spermine

Abbreviations: HTS – high temperature stress; PA – polyamines; Put – putrescine, Spd – spermidine, Spm – spermine

Introduction. Aliphatic polyamines are ubiquitous amines synthesized in both prokaryotic and eukaryotic organisms [1]. In plants, they exist in both free and bound forms. There are two classes of polyamines bound either to low-molecular-mass (TSA-soluble) or to high-molecular-mass (TCA-insoluble) compounds [2]. Accumulation of polyamines has been shown under a variety of stress factors, including high salinity,

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BIOLOGIE
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INFLUENCE OF 4PU-30 AND THIDIAZURON ON HYDROGEN PEROXIDE AND SOME DEFENCE ENZYMES OF IN VITRO CULTURED APPLE AND PEACH[1]

I. Sergiev, D. Todorova, K. Kornova*, V. Alexieva, E. Karanov

(Submitted on April 20, 2005)

Abstract

The effect of the phenylurea cytokinins 4PU-30 and thidiazuron (TDZ) on hydrogen peroxide levels, and peroxidase, catalase and IAA-oxidase activity on in vitro cultured apple and peach was studied. Explants of apple (rootstock MM 106) and peach (rootstock GF 677) were cultured on solid Murashige-Skoog medium. Both compounds were added to the standard medium and tested at concentrations of 0.5, 0.05, 0.005 and 0.0005 mg/l. The higher concentrations increased the activity of peroxidase and catalase. The effect observed diminished with the decrease of the concentration applied. Both compounds enhanced endogenous level of hydrogen peroxide. However, both plant growth regulators had opposite effect in relation to IAA-oxidase activity, i.e. TDZ enhanced IAA-oxidase activity, while 4PU-30 induced inhibition with exception of concentration 0.5 mg/l.

Key words: in vitro culture, plant growth regulators

Abbreviations: 4PU-30 – N^1-(2-chloro-4-pyridyl)-N^2-phenylurea; BAP – N^6-benzylaminopurine; TDZ – N^1-(1,2,3-thiadiazol-5-yl)-N^2-phenylurea

Introduction. The mechanisms and principles of governing organ formation or various morphogenetic phenomena of higher plants are of great interest. The cultivation of plants in vitro provokes a significant physiological shock generally due to the disturbance of the phytohormone biosynthesis and distribution [1]. Plant growth regulators, especially auxins and cytokinins, are obligatory components of the nutrient medium used for in vitro cultivation. Kinetin and N^6-benzylaminopurine (BAP) are much more widely used than the other purine cytokinins. The ability of some phenylureas to substitute cytokinin active derivatives was demonstrated in several plant systems. N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4PU-30, CPPU) and other related compounds were found to be more active than purine cytokinins [2,3], but because they are not readily available, they are still not extensively used. Thidiazuron [N^1-(1,2,3-thiadiazol-5-yl)-N^2-phenylurea, TDZ], commercially used as a cotton defoliant at least in part because

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BIOLOGIE
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EFFECT OF SOME PLANT GROWTH REGULATORS ON PHYSIOLOGICAL AND BIOCHEMICAL STATUS OF IN VITRO MICROPROPAGATED PLANTLETS FROM APPLES DURING ADAPTATION

D. Todorova, I. Sergiev, K. Kornova*, V. Alexieva, E. Karanov

(*Submitted on May 25, 2005*)

Abstract

The effect of the phenylurea cytokinin 4PU-30, gibberellic acid (GA_3) and natural (Sm) and synthetic (DETA) polyamines on some stress markers' content, enzymatic activity and free polyamine levels in two in vitro cultured apples (rootstocks MM106 and M26) was studied. All compounds were leaf applied to plants during adaptation period of rooted plantlets. The treatment with these plant growth regulators provides a promising opportunity for adaptation of apples in non-sterile medium and improves their physiological and biochemical status. The compounds applied reduce the stress-provoked damages due to the transition from in vitro to in vivo growth conditions.

Key words: adaptation, in vitro culture, plant growth regulators

Abbreviations: 4PU-30 - N^1-(2-chloro-4-pyridyl)-N^2-phenylurea; CAT – catalase, DETA – diethylenetriamine, GA_3 – gibberellic acid, MDA - malondialdehyde, LSD – low significant difference, POD - guaiacol peroxidase, Pt – putrescine, Sd – spermidine, Sm – spermine, SOD – superoxide dismutase

Introduction. Adaptation of in vitro microprapagated plantlets is a final process of cultivation when plants pass from in vitro to in vivo growth conditions. Successful adaptation is a limited factor for commercial practice. Usually the cultivation of plants in vitro provokes a significant physiological shock. When plantlets are through to in vivo growth there is an additional plant stress. The treatment with some plant growth regulators and low positive temperature (4 °C) may help overcome plant dormancy and reduce stress during adaptation period [1–7]. All these works studied only the physiological effect of the application of different plant growth regulators. However, there is no information about regulatory mechanisms of the applied compounds. That is why we have made more detailed investigations in order to obtain additional information about the origin of this kind of stress and to study the possibilities for overcoming the damages provoked by stress with the compounds with proved antistress activity – phenylurea cytokinin, native and synthetic polyamines. The purpose of the experi-

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BIOLOGIE
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CYTOKININ POOL DYNAMIC CHANGES AND DISTRIBUTION OF CYTOKININ OXIDASE/DEHYDROGENASE ACTIVITY IN PEAS IN RELATION TO DEVELOPMENTAL SENESCENCE

I. Vaseva-Gemisheva, D. Todorova, J. Malbeck*, A. Travnickova*, I. Machackova*, E. Karanov

(*Submitted on October 19, 2005*)

Abstract

Variable distribution of cytokinin oxidase/dehydrogenase activity (CKX: EC 1.5.99.12) in leaves of young pea seedlings (cv. Manuela) during the period needed for full leaf bud expansion was observed. Lower iPR/iPRP and *cis*ZR/*cis*ZRP contents were detected at stages characterised by higher CKX activity. Respective bases were poorly represented in the leaf tissue in this model system during the whole experimental period. *Cis*Z and its derivatives were found to be the dominant form of Z-type cytokinins at this particular stage of development in pea. CKX activity correlated with the changes in cytokinin pool which may have triggered developmental events in leaves during bud expansion. These changes followed a regular pattern characterised by two peaks of enzymatic activity: the first one at stages corresponding to active organ development (with subsequent decrease of CKX in fully expanded leaves), and the second one related to beginning of senescence processes in the oldest leaves. The data suggest that CKX-mediated dynamic changes in cytokinin pool participate in internal control mechanisms of developmental senescence.

Key words: cytokinins, cytokinin oxidase/dehydrogenase, developmental senescence, *Pisum sativum*

Abbreviations: BSA – bovine serum albumin, *cis*Z – *cis* zeatin, *cis*ZR – *cis* zeatin riboside, *cis*ZRP – *cis* zeatin riboside monophosphate, CK – cytokinins, CKX – cytokinin oxidase/dehydrogenase, HPLC – high performance liquid chromatography, iP – isopentenyl adenine, iPR – isopentenyl adenine riboside, iPRP – isopentenyl adenine riboside monophosphate

Introduction. Leaf senescence is a programmed process and each individual leaf has a similar lifespan. The best evidence for hormonal involvement in the control of senescence is for cytokinins and ethylene [1]. Ethylene will cause rapid senescence of older leaves. However, the effect of ethylene is strictly dependent on developmental signal(s), and this hormone does not affect young leaves [1]. Cytokinins are probable candidates for hormones involved in age-dependent senescence processes [2]. Endogenous cytokinin content seems to be one of the important parameters defining plant organ

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INFLUENCE OF ETHYLENE RELEASING AGENT ETHREL ON CYTOKININ OXIDASE/DEHYDROGENASE ACTIVITY IN TISSUES OF *PISUM SATIVUM*

I. Vaseva-Gemisheva, D. Todorova, E. Karanov

(*Submitted by Academician V. Golemansky on January 25, 2006*)

Abstract

Ethylene releasing agent Ethrel was found to influence positively the total cytokinin oxidase/dehydrogenase activity (CKX, EC 1.5.99.12) in leaves and roots of young *Pisum sativum* plants (cv. Manuela). Certain relationship between the distribution of the enzymatic activity in the different tissues and the applied concentration of Ethrel was established. Low concentrations (0.5 and 1.0 μM) of the ethylene producer provoked increase of CKX in roots while the higher ones (10 and 15 μM) resulted in elevated enzymatic activity in the last fully expanded leaf pair. Results suggest that higher Ethrel concentrations accelerate senescence processes in leaf tissue which is accompanied with increase of CKX. This is probably due to the intensive metabolism of substrate cytokinins transported from roots to shoots.

Key words: cytokinins, cytokinin oxidase/dehydrogenase, ethylene, Ethrel, *Pisum sativum*

Abbreviations: BSA – bovine serum albumin, CKX – cytokinin oxidase/dehydrogenase

Introduction. It has been found that ethylene largely mediates a number of responses to exogenous cytokinin in Arabidopsis, such as the inhibition of root and hypocotyl elongation [1, 2]. Many of the morphogenic changes produced by cytokinin in Arabidopsis seedlings are similar to those that result from the action of ethylene [3]. Several studies indicate that there is a relationship between the so-called *KNOX* genes and the signal transduction pathways of different hormones [4]. *KNOX* genes code for homeodomains of important transcriptional factors which are responsible for the proper function of plant meristems and organ formation [5]. It has been established [6] that ethylene and cytokinins act antagonistically regarding regulation of meristemic activity via group of genes which code for factors regulating cell division. Plant development is modulated by interactions of hormones, illustration of which is induction of biosynthesis of one hormone in response to another. N^6-substituted adenine cytokinins are one of the factors that modulate biosynthesis of ethylene [7–9]. Cytokinins elevate ethylene biosynthesis in etiolated Arabidopsis seedlings via a posttranscriptional modification of one isoform of the key biosynthetic enzyme 1-aminocyclopropane 1-carboxylic acid

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EFFECTS OF SOME PLANT GROWTH REGULATORS ON THE ENDOGENOUS POLYAMINE CONTENT IN CULTIVATED IN VITRO *ROSA HYBRIDA* L., CV. MADELON

D. Todorova, V. Kapchina-Toteva*, E. Yakimova**, I. Sergiev, V. Alexieva, E. Karanov

(*Submitted on December 22, 2004*)

Abstract

The effects of the auxin indolylbutyric acid, cytokinins N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4PU-30) and N^6-benzyladenine, as well as the cytokinin antagonists 2-chloro-4-cyclobutyl-amino-6-ethylamino-1,3,5-triazine and N-(4-pyridyl)-O-(4-chlorophenyl)-carbamate on the amount of free, TCA-soluble and TCA-insoluble polyamines in in vitro cultured one-month-old plantlets of *Rosa hybrida* L., cv. Madelon were studied. Single nodes from shoot cultures were grown on standard MS medium supplemented with the tested plant growth regulators. Elevated amounts of spermine, spermidine and putrescine were detected after 4PU-30 application. In benzyladenine-treated plantlets, the levels of polyamines were reduced as compared to the control plants. An exception was observed in TCA-soluble forms of polyamines. The application of indolylbutyric acid caused a decrease of free and TCA-insoluble putrescine and spermine, but enhanced the same fractions of spermidine and all TCA-soluble forms. Accumulation of polyamines was detected after anticytokinin application. The obtained results suggest an existing physiological effect of the used anticytokinins on the endogenous polyamine levels.

Key words: anticytokinins, auxin, cytokinins, polyamines, putrescine, spermidine, spermine

Abbreviations: 4PU-30 – N^1-(2-chloro-4-pyridyl)-N^2-phenylurea; ACK1 – 2-chloro-4-cyclobutyl-amino-6-ethylamino-1,3,5-triazine; ACK2 – N-(4-pyridyl)-O-(4-chlorophenyl)-carbamate; BA – N^6-benzyladenine; IBA – indolylbutyric acid; Put – putrescine; Spd – spermidine; Spm – spermine; TCA – trichloroacetic acid; TLC – thin layer chromatography

Introduction. Polyamines are aliphatic amines which are ubiquitous for plants, bacteria, fungi and all cell types examined so far, and putrescine, spermidine and spermine are common to all plant species [1]. Polyamines appear to be involved in

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EFFECT OF ENHANCED UV-C IRRADIATION ON THE GROWTH, MALONDIALDEHYDE, HYDROGEN PEROXIDE, FREE PROLINE, POLYAMINES, IAA AND IAA-OXIDASE ACTIVITY IN PEA PLANTS (*PISUM SATIVUM* L.)

Zornitsa Katerova, Dessislava Todorova

(*Submitted by Academician A. Atanassov on June 20, 2011*)

Abstract

The effect of low dose (LD) of 0.1 kJ m^{-2} d^{-1} and high dose (HD) of 0.3 kJ m^{-2} d^{-1} of UV-C irradiation on some growth parameters (aboveground length, fresh and dry weight), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), free proline, free, conjugated and bound spermine (Spm), spermidine (Spd) and putrescine (Put), free indole-3-acetic acid (IAA) content and IAA-oxidase activity in leaves of pea plants after 21 days of consecutive treatment was studied. As it was expected all measured growth parameters were reduced in dose-dependent manner. LD regime does not influence stress markers content. HD regime led to insignificant change in proline amount, some increase in H_2O_2 content (assuming signal role), and considerable decrease in MDA amount. UV-C treatments cause substantial increase of bound PA-fractions, which correlated negatively with reduced MDA and indicate lower lipid peroxidation of unsaturated fatty acids of biomembranes. A reduction of free PAs (except Spm) was found after both UV-C regimes. Drop in conjugated PAs concentration was observed only in Spd after LD regime. Alterations in free and conjugated PAs pool could be due to conversion of free into conjugated form. It was determined that HD regime lessen IAA content, while LD regime had an opposite effect. Possibly IAA changed specifically to different doses of UV-C irradiation without participation of IAA-oxidase as its activity was insignificantly altered.

This study reinforces previous reports with the same model system and shorter treatment periods (7 and 14 d LD and HD). On the basis of the presented here results we assume that the additional period of UV-C irradiation facilitates pea to improve repair processes under prolonged low intensive UV-C treatment.

Key words: UV-C radiation, pea, polyamines, IAA, IAA-oxidase, hydrogen peroxide, malondialdehyde, proline

Abbreviations: HD – high dose UV-C, H_2O_2 – hydrogen peroxide, IAA – indole-3-acetic acid, LD – low dose UV-C, MDA – malondialdehyde, PAs – polyamines, Put – putrescine, Spd – spermidine, Spm – spermine, UV – ultraviolet radiation

Introduction. Ultraviolet radiation is classified as UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). UV-C quickly creates high levels of damage, more than UV-B, because of its highest energy [1]. Generally, UV-C

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BIOLOGIE

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POLYAMINES AND FREE PROLINE PROTECT YOUNG PEA (*PISUM SATIVUM* L.) LEAVES AGAINST ENHANCED UV-C IRRADIATION

Zornitsa Katerova, Dessislava Todorova

(*Submitted by Academician A. Atanassov on November 21, 2011*)

Abstract

Pea (*Pisum sativum* L.) seedlings were exposed to short pulses of ultraviolet-C (UV-C) radiations: low dose (LD – 0.1 kJ m^{-2} d^{-1}) and high dose (HD – 0.3 kJ m^{-2} d^{-1}). Concentrations of malondialdehyde (MDA); hydrogen peroxide (H_2O_2); free proline; free, conjugated and bound spermine (Spm), spermidine (Spd) and putrescine (Put) were determined in 4th leaves of pea plants after 21 consecutive days of UV-C treatment. Free proline, H_2O_2 and MDA did not change markedly after LD regime. HD regime led to a significant increase in free proline and H_2O_2 amount, accompanied by a considerable decrease in MDA content. HD treatment causes a substantial increase in bound PA-fractions. Any significant changes were found in bound fraction after LD regime. Alterations in free and conjugated PAs pool provoked by both UV-C treatments could be due to conversion of free into conjugated form and vice versa. HD regime provokes adaptation processes in 4th leaves: the augmentation of free proline and bound PAs protects cell membranes against prolonged low intensive UV-C radiation. This confirms that endogenous PAs as well as proline have an important role in plant defence responses under short pulses of UV-C treatment.

Key words: UV-C radiation, pea, polyamines, hydrogen peroxide, malondialdehyde, proline

Abbreviations: HD – high dose UV-C, H_2O_2 – hydrogen peroxide, LD – low dose UV-C, MDA – malondialdehyde, PAs – polyamines, Put – putrescine, ROS – reactive oxygen species, Spd – spermidine, Spm – spermine, UV – ultraviolet radiation

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Oxidative stress in biological systems

OXIDATIVE STRESS PROVOKED BY LOW AND HIGH TEMPERATURES IN WILD TYPE AND ETHYLENE-INSENSITIVE MUTANT *ETI5* OF *Arabidopsis thaliana* (L.) H e y n h

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ABSTRACT

Arabidopsis thaliana (L.) H e y n h wild type (*wt*) and ethylene-insensitive (*eti5*) type plants were used in this study. The plants were grown in growth chamber and 38 days after sowing the plants were subjected to low temperature (LT) 4°C or high temperature (HT) 38°C for 24 h in darkness. The content of stress markers and enzyme activities were measured at 0, 24, 48 and 120 h after the temperature treatment. The aim of our investigation was to compare the effect of low and high temperature on hydrogen peroxide (H_2O_2), malondialdehyde (MDA), free proline and carbonyl group content, ascorbate/dehydroascorbate content as well as catalase, guaiacol peroxidase (POD), and superoxide dismutase (SOD) activities in both types of *Arabidopsis.* Data obtained showed higher levels of the stress-markers MDA and carbonyl groups as well as decreased catalase activity (detoxifying H_2O_2) and increased SOD activity (producing H_2O_2) at the end of the measuring period (120 h) in the *wt* than in the mutant, which indicates that the *wt* is more sensitive to temperature stress than the mutant. On the other hand, the observed higher levels of stress markers (carbonyl groups, MDA) in both genotypes at 0 h after HT treatment as compared to LT is indicative that *Arabidopsis* is more sensitive to HT stress probably due to the fact that this plant species is cold-tolerant.

Keywords: *Arabidopsis thaliana* (L.) H e y n h, ethylene-insensitive mutant (*eti5*), temperature stress, stress markers.

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POLYAMINE SPERMINE PROTECTS YOUNG PEA PLANTS AGAINST ULTRAVIOLET-C RADIATION

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Abstract
The effects of ultraviolet-C (UV-C) irradiation and the polyamine spermine on the contents of some stress marker and non-enzymatic antioxidants in leaves of young pea were investigated. UV-C irradiation led to decrease in pea fresh weight, content of leaf pigments, free proline, accompanied with increase in malondialdehyde. Initial augmentation in free thiol levels was transient in UV-C treated plants and finally a substantial lessening was found. Spermine led to a significant augmentation of free thiols and proline content along with a decline in total phenols, but these alterations diminished during the experimental period. On the base of comparative analyses of the results obtained for plants treated with UV-C and polyamine it could be concluded that the preliminary application of spermine protects pea plants against irradiation by maintaining normal plant growth, stabilizing cell membranes and activating non-enzymatic antioxidants.

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ОБЗОРИ

ЕТИЛЕНОВИ МУТАНТИ НА ARABIDOPSIS THALIANA L. *HEYNH*

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Резюме: *В статията е направен опит да се обобщи известната до момента информация за етиленовите мутанти на Arabidopsis thaliana, които са част от изключително голямото разнообразие от растителни мутанти на този вид въобще. Посочени са основните класове етиленови мутанти и са дадени някои по-важни техни характеристики. Представени са и основните компоненти от пътя за приемане и пренасяне на сигнала за етилена – основен хормон в растенията и един от главните фитохормонални регулатори на стареенето, което е пряко свързано с продуктивността на растенията.*
Ключови думи: *Arabidopsis thaliana, етиленови мутанти, етиленов сигнал, троен отговор*

D. TODOROVA, Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia. ***ETHYLENE MUTANTS OF ARABIDOPSIS THALIANA* L. *HEYNH***

Abstract*: In the present review an attempt has been made to summarize the available information about ethylene mutants that are a part of the enormous variety of Arabidopsis thaliana mutants. The main classes of mutants together with some of their more important characteristics are listed here. The basic components of ethylene signal perception and transduction pathway are also discussed. Ethylene is one of the major phyto-hormones which participates in the regulation of senescence. The senescence is close related to growth efficiency and plant production.*
Key words: *Arabidopsis thaliana, ethylene mutants, ethylene signal, triple response*

Растителни мутанти по хормони

Фитохормоните повлияват множество процеси на растежа и развитието на растенията – от образуването на зародиш в семената до формирането на корени, стъбла и цветове. От друга страна, всеки фитохормон в повечето случаи проявява своето физиологично и регулаторно действие в неразривна връзка с другите растителни хормони. Например в растението един единствен хормон може да регулира множество различни процеси и в същото време различни хормони да повлияват един единствен процес (McCourt, 1999).

Развитието на молекулярната биология и приложението ѝ в изучаване на действието на фитохормоните е свързано в последните години с използването на растителни мутанти.

Първите мутанти, използвани в изследванията за промените в растителните хормони, притежават определен фенотип, лесно различим в зависимост от ефекта на определени хормони.

Най-широко прилаганият метод за получаване на хормонални мутанти е използване на химични мутагени или йонизираща енергия. Успехът при прилагането на тези техники зависи от качеството

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TWO PEA VARIETIES DIFFER IN CYTOKININ OXIDASE/ DEHYDROGENASE RESPONSE TO UV-B IRRADIATION

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Summary. Cytokinin oxidase/ dehydrogenase (CKX EC: 1.5.99.12) and cytokinin changes after UV-B irradiation (302 nm) in two pea varieties with different vegetation periods have been studied. Treatment caused total inhibition of CKX and reduced endogenous cytokinins in the slower-growing "Manuela", while it induced the enzymatic activity and positively influenced hormonal content in leaves of the faster-growing cultivar – "Scinado". Results suggest presence of diverse *ckx* alleles in the genomes of both varieties, which are characterized with different basal endogenous cytokinin concentration.

Key words: cytokinins, cytokinin oxidase/ dehydrogenase, *Pisum sativum*, UV-B.

Abbreviations: BSA – bovine serum albumin, *cis*Z – *cis* zeatin, *cis*ZR – *cis* zeatin riboside, *cis*ZRP – *cis* zeatin riboside monophosphate, CK – cytokinins, CKX – cytokinin oxidase/ dehydrogenase, HPLC – high performance liquid chromatography, iP – isopentenyl adenine, iPR – isopentenyl adenine riboside, iPRP – isopentenyl adenine riboside monophosphate, PMSF – phenylmethylsulfonyl fluoride.

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