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*BIOLOGIE*
*Physiologie des plantes*

# 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.

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# 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.

BIOLOGIA PLANTARUM 48 (4): 593-595, 2004

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 (6$^{th}$ and 7$^{th}$ 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.

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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

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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.

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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.

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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.

Plant Growth Regulation (2005) 46:193–197
DOI 10.1007/s10725-005-0650-2


# 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.

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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

*Dessislava Todorova[1*], Irina Vaseva-Gemisheva[1], Petar Petrov[1], Ekaterina Stoynova-Bakalova[1], Vera Alexieva[1], Emanuil Karanov[1], Aileen Smith[2], Michael Hall[2]*

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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.

adenosine, *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)

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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.

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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.

BIOLOGIA PLANTARUM 51 (2): 377-380, 2007

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 ($3^{rd}$ to $7^{th}$ 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.

BIOLOGIA PLANTARUM 51 (3): 579-583, 2007

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.

Plant Growth Regul (2007) 51:185–191
DOI 10.1007/s10725-006-9143-1

ORIGINAL PAPER

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

**Dessislava Todorova · Iskren Sergiev · Vera Alexieva · Emanuil Karanov · Aileen Smith · Michael Hall**

Received: 26 January 2006 / Accepted: 18 October 2006 / Published online: 1 February 2007


**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 |

Acta Physiol Plant (2008) 30:151–155
DOI 10.1007/s11738-007-0103-9

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**

Received: 15 March 2007 / Revised: 21 August 2007 / Accepted: 18 September 2007 / Published online: 6 October 2007


**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.

## 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

Plant Growth Regul (2009) 57:145–152
DOI 10.1007/s10725-008-9330-3

ORIGINAL PAPER

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

**Zornitsa I. Katerova · Dessislava Todorova**

Received: 5 February 2008 / Accepted: 18 September 2008 / Published online: 1 October 2008


**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.

**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 |

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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

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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.

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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.

# POLYAMINE SPERMINE PROTECTS YOUNG PEA PLANTS AGAINST ULTRAVIOLET-C RADIATION

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## ABSTRACT

*The effects of ultraviolet-C (UV-C) irradiation and polyamine spermine on the content of some stress marker and non-enzymatic antioxidants in leaves of young pea plants were investigated. UV-C irradiation led to a decrease in pea fresh weight, the content of leaf pigments and free proline, accompanied with an increase in malondialdehyde. The initial augmentation in the free thiol levels was transient in UV-C treated plants and finally a substantial decrease 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. Based on comparative analyses of the results obtained for plants treated with UV-C and polyamine, it could be concluded that 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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*Oxidation Communications 38, No 1, 104–113 (2015)*

*Antioxidants, antiradical agents, inhibitors*

# ENDOGENOUS POLYAMINE PROFILES OF ISOLATED ZUCCHINI COTYLEDONS INCUBATED ON SOLUTIONS OF $Cu^{2+}$ AND METHYL JASMONATE

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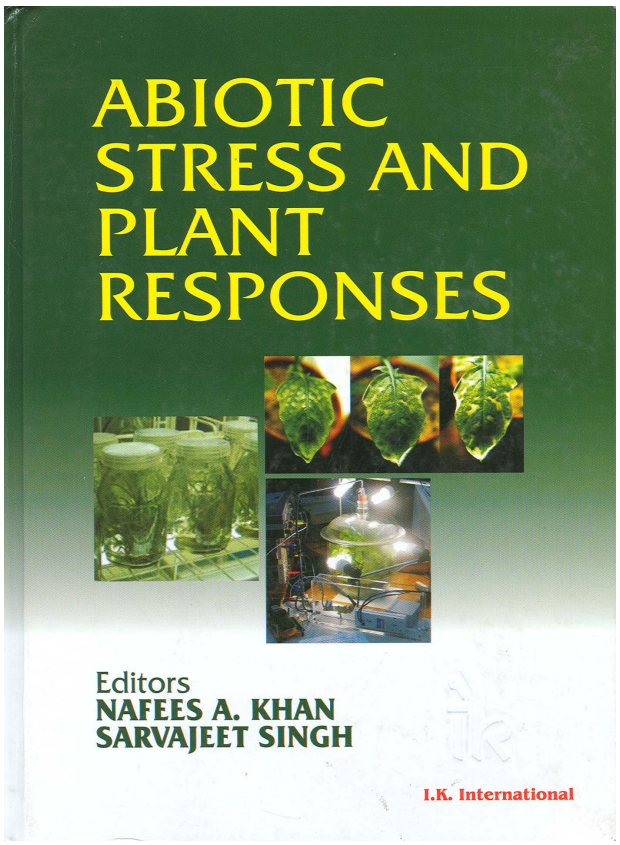
ABSTRACT

Polyamines putrescine, spermidine, and spermine possess hormone-like features and are involved in the regulation of plant growth and developmental processes. In our study, we determined the changes in the endogenous concentrations of putrescine, spermidine and spermine and attempted to assess the role of polyamines in the growth of *Cucurbita pepo* cotyledons incubated on solutions containing growth factors with inhibitory effect on plant cell division – methyl jasmonate and copper. We confirm that endogenous polyamines (and mainly conjugated putrescine and spermidine) are involved in cell division processes of isolated zucchini cotyledons and their role is implicated in overcoming the inhibition of cell growth provoked by growth regulating factors tested.

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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

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## 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.

# 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).

### *13.1.1 Reactive Oxygen Species*

Independently of the type of stress (natural or anthropogenic), the accumulation of ROS is an undeniably established fact. Currently, overproduction of more than ten

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# 11 Polyamines – Involvement in Plant Stress Tolerance and Adaptation

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## 11.1 Polyamines – Occurrence, Chemistry and Metabolism

The triamine spermidine (Spd), tetraamine spermine (Spm) and their precursor the diamine putrescine (Put) are the major polyamines (PAs) widespread in all plant species. Polyamines are organic compounds with low-molecular weight and a straight-chain $C_3$-$C_{15}$ aliphatic structure that includes at least two primary amino groups and one or more internal imino groups (Edreva, 1996; Groppa and Benavides, 2008; Gill and Tuteja, 2010a). Besides Put, Spd and Spd, which are common for all plant species, there are also unusual PAs occurring only in distinct plant species (i.e. diamines cadaverine and 1,3-diaminopropane, and PAs homospermine, thermospermine and canavalmine), or synthesized under certain conditions, i.e. norspermine and norspermidine (Table 11.1).

Polyamine biosynthesis in plants can be outlined as a two-stage process (Fig. 11.1) – the first phase is the biosynthesis of diamines, and the second phase is Spd and Spm biosynthesis. The Put is synthesized through decarboxylation of L-arginine to agmatine by arginine decarboxylase (ADC), followed by hydrolysis and deamination of agmatine by agmatine iminohydrolase and formation of N-carbamoylputrescine. Further, N-carbamoylputrescine is subjected to hydrolysis, deamination and decarboxylation by N-carbamoylputrescine amidohydrolase to outcome the final product Put. An alternative pathway for Put synthesis is the direct decarboxylation of L-ornithine, catalysed by ornithine decarboxylase (ODC). Spermidine and Spm are synthesized by incorporation of an aminopropyl residue from decarboxylated S-adenosylmethionine to Put or Spd – this step is catalysed by the enzymes spermidine synthase (SPDS) or spermine synthase (SPMS) respectively. The essential for PAs biosynthesis, decarboxylated S-adenosylmethionine, is formed by decarboxylation (S-adenosylmethionine decarboxylase, SAMDC) of S-adenosylmethionine, which is a common precursor of PAs and ethylene (Slocum, 1991).

The PA degradation (Fig. 11.2) is realized through oxidative deamination catalysed by aminooxidases. They are copper-containing diamine oxidases (DAO) and flavoprotein-containing polyamine oxidases (PAO). DAO oxidize the primary amino groups of PAs. The oxidative deamination of Put produces $\Delta^1$-pyrroline, $H_2O_2$ and $NH_3$. PAO oxidize the secondary amino groups of PAs and the final products of the process are $\Delta^1$-pyrroline (from Spd oxidation) or

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# *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.*

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*DOI: 10.1556/AAgr.57.2009.1.4*

# 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.

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*DOI: 10.1556/AAgr.60.2012.1.1*

# 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.

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Scientific Research

# Polyamine Alterations in Isolated Zucchini Cotyledons Grown in Presence of Cytokinins and $Cu^{2+}$

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## Abstract

**Polyamines are small nitrogen-containing organic molecules, which are widely distributed in plants. They are involved in the regulation of normal plant growth and developmental processes. In this study we examined the role of polyamines on the growth of *Cucurbita pepo* L. (zucchini) cotyledons incubated on solutions of different types of cytokinins (BA—$N^6$-benzylaminopurine or 4PU-30—$N^1$-(2-chloro-4-pyridyl)-$N^2$-phenylurea) and copper in excess. We found that endogenous polyamines, and mainly the conjugated fraction, are involved in the cell division processes of isolated zucchini cotyledons and their changes are related to the specific action of the used growth regulating factors.**

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Scientific Research

# Protective Effect of Hydrogen Peroxide against Paraquat Toxicity in Young Pea Plants: Possible Role of Endogenous Polyamines

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## Abstract

**Young pea plants grown as a water culture were sprayed with hydrogen peroxide and 24 h later were treated with the herbicide paraquat. The effects of paraquat and $H_2O_2$ treatment were investigated on the endogenous concentrations of free proline, and free and conjugated putrescine, spermidine and spermine. Application of PQ increased the free proline and Put, and decreased conjugated and bound polyamines. The results clearly demonstrate the oxidative damages provoked by PQ application. Single treatment with $H_2O_2$ provoked considerable decrease in the free fraction of the three PAs studied 2 h after light exposition, but caused an increase in their respective conjugated fractions. Data suggest that PA changes after $H_2O_2$ treatment are due to conversion from free to conjugated form. Three hours later free PAs in $H_2O_2$ treated-plants reached control levels; however the conjugated remained higher than the control. No alterations were detected in proline concentration after $H_2O_2$ treatment. Pretreatment with $H_2O_2$ activated some component of the plant protection mechanisms by causing an alteration in free/conjugated PAs ratio in plants subsequently subjected to PQ treatment. Data concerning PA and proline concentrations in plants treated with both compounds supposed that $H_2O_2$ shows a protective role against PQ and improves the plant tolerance to the oxidative stress generated by paraquat.**

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# CHANGES OF ENDOGENOUS POLYAMINES IN EXCISED *CUCURBITA PEPO* L. (ZUCCHINI) COTYLEDONS CULTIVATED IN THE PRESENCE OF BENZYLADENINE AND METHYL JASMONATE

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Received: *23 October 2014* Accepted: *09 January 2015*

**Summary:** In the present work, we studied the changes in the content of polyamines putrescine (Put), spermidine (Spd), and spermine (Spm) in *Cucurbita pepo* L. (zucchini) cotyledons excised from dry seeds and incubated in darkness on solutions of $N^6$-benzylaminopurine (BA) or methyl ester of jasmonic acid (MeJA) each applied at a concentration of 0.01mM. Putrescine content was altered most significantly, followed by less considerable amendments of Spd content. No changes of Spm amount in MeJA-treated cotyledons were detected. BA provoked an increase in free polyamines which was accompanied by the 'disappearance' of the conjugated forms as well as abundance of bound polyamines. The changes of endogenous polyamines are discussed in terms of the specific action of the tested phytohormones on cell proliferation and confirm the important role of polyamines in cell division processes in excised zucchini cotyledons.

Genetics and Plant Physiology – 2015, Volume 5(2), pp. 123–144

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Review

# POLYAMINES – POSSIBILITIES FOR APPLICATION TO INCREASE PLANT TOLERANCE AND ADAPTATION CAPACITY TO STRESS

***Todorova D.**, Z. Katerova, V. Alexieva, I. Sergiev***

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Received: *22 November 2014* Accepted: *23 January 2015*

**Summary:** Polyamines are plant growth regulators distributed among all plant species. The major polyamines are diamine putrescine, triamine spermidine and tetraamine spermine. Polyamines are involved in a number of growth and developmental processes, as well as in plant responses to a variety of unfavorable factors. Polyamines can stabilize cell constituents and protect macromolecules from free radicals generated by adverse stress agents. Endogenous polyamines could contribute to plant stress tolerance as part of defense mechanisms or adaptation programs that help plant organism to cope with the negative stress consequences. The current review is focused on the latest investigations regarding the possibilities for exogenous polyamine application to increase plant tolerance and adaptation capacity to diverse abiotic impacts including mineral nutrient deficiency, salinity, water stress, heavy metal toxicity, low and high temperature stresses, UV irradiation, ozone, air pollutants and herbicides as well as to biotic stress factors such as infections with fungal, bacterial and viral pathogens and attacks by herbivorous insects.

*Gen. Appl. Plant Physiology, Special Issue, 2006,131-138* 131

# 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.