

## MINIREVIEW

## Cytokinins and water stress

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## Abstract

It is almost impossible to find a single process in plant life that is not affected by both stress and hormones directly or indirectly. This minireview is focused on the interactions between water stress and cytokinins (CKs). The attention was paid mainly to changes in endogenous CK content and composition under water stress, involvement of CK in plant responses to water stress mainly in stomatal regulation of gas exchange, water relations of transgenic plants with elevated CK content, and possibilities to ameliorate the negative effects of water stress by application of exogenous CKs.

*Additional key words:* abscisic acid, chlorophyll, isopentenyladenine, isopentenyladenosine, leaf water potential, net photosynthetic rate, stomatal conductance, transpiration rate, zeatin, zeatin riboside.

## Introduction

Although water is the most abundant molecule on the earth surface, the availability of water strongly restricts terrestrial plant production. Thus, if we want to increase productivity of agriculture and forestry we need to understand controls over plant water relations and consequences of water stress. In addition to drought, water stress can be induced by salinity, low temperature, pathogens, etc. Different mechanisms were developed during plant evolution to prevent dangerous water loss, and to tolerate a mild one. Shoot desiccation is avoided mainly by stomatal regulation of transpiration rate. In response to water stress a shift in a relationship between water and pressure potential has been observed. This can be reached by osmotic adjustment (accumulation of osmotically active substances), increased cell wall elasticity, and/or increased apoplastic water fraction. A number of genes that respond to water stress have been

recently described and stress-induced proteins identified. Under water stress, reactive oxygen species might be generated and the activation of defence mechanisms against them is the further important component of stress-protective mechanism (for review see, e.g., Levine 1999).

In many cases, water deficit reduces growth, and leaf area development and duration. Stomatal closure decreases the CO<sub>2</sub> influx which limits photosynthesis under mild water stress and supports photoinhibition under high irradiance. The severe water stress directly affects photosynthetic capacity of the mesophyll causing decrease in carboxylation as well as electron transport chain activities, and/or induces ultrastructural changes in chloroplasts. Evolution of CAM and C<sub>4</sub> photosynthetic pathways contribute to better survival and productivity of these species under water stress. Water stress affects many metabolic pathways, mineral uptake, membrane

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*Abbreviations:* ABA - abscisic acid; BAP - 6-benzylaminopurine; Chl - chlorophyll; CK - cytokinin; (diH)Z - dihydrozeatin; (diH)ZR - dihydrozeatin riboside; d.m. - dry mass; E - transpiration rate; f.m. - fresh mass; g<sub>s</sub> - stomatal conductance; iP - isopentenyladenin; iPA - isopentenyladenosine; Kin - kinetin; P<sub>N</sub> - net photosynthetic rate; RWC - relative water content;  $\psi_w$  - leaf water potential; Z - zeatin; ZR - zeatin riboside.

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structure, *etc.* Therefore it is not surprising that also hormone content can be changed. It is very important because plant hormones are considered as main signals in root-to-shoot communication and *vice versa* (for review see, *e.g.*, Davies and Zhang 1991, Tardieu and Davies 1993, Davies 1995, Naqvi 1995). In consequence, the change of hormonal balance might play the key role in the sequence of events induced by stress (Itai 1999). Despite of this, the mechanism of induction of a hormonal shift by an environmental change is unknown.

Cytokinins (CKs) promote cell division and, acting both in synergy and antagonism with other plant hormones, influence a wide range of events during plant growth. The major portion of CKs are produced in meristematic regions in the root system and transported via xylem to the shoot. These CKs, along with the locally synthesized CKs, control development and senescence of the whole plant. CKs promote leaf expansion, accumulation of chlorophyll and conversion of etioplasts into chloroplasts, and delay leaf senescence. The molecular mechanism of CK action is only poorly understood and an information how endogenous CKs are affected under stress is meager. In the light of the important regulatory role played by CKs in modulating

development, it seems feasible also to anticipate their involvement in response to adverse environmental conditions (Hare *et al.* 1997, Brault and Maldiney 1999).

Plant responses to CKs are often judged from their responses to exogenously applied CKs. However, when we apply CKs and follow the plant response, it is necessary to take into consideration that exogenous CKs (natural and synthetic) can increase content of endogenous CKs by their uptake and by promotion of CK biosynthesis. On the other hand, they can increase cytokinin oxidase activity and CK degradation (for review see, *e.g.*, Hare *et al.* 1997, Kaminek *et al.* 1997). Thus, the composition and concentration of CKs in the site of action might be quite different than in the site of application.

In this minireview, we aim to illustrate 1) changes in endogenous cytokinins induced by water stress, 2) their involvement in plant responses to water stress mainly in stomatal regulation of gas exchange, 3) possibilities of amelioration the negative effects of water stress by application of exogenous cytokinins, and 4) water relations of transgenic plants with elevated cytokinin content.

## Changes in endogenous cytokinins induced by water stress

Xylem exudate and/or leaves of stressed plants usually exhibit reduced CK content and activity. The response is usually rapid and CK activity returns to a normal level after a release of stress (for review see, *e.g.*, Naqvi 1994, 1995). The explanation for the detection of reduced CK content under water stress is either a reduction in CK biosynthesis or enhanced degradation.

Recently, decreased content of CKs was found in alfalfa under drought (Goicoechea *et al.* 1995, 1997). This CK content decrease was accompanied with accelerated senescence. The effects were less pronounced in plants inoculated with *Glomus* or *Rhizobium* than in controls. The contents of Z, zeatin riboside (ZR), isopentenyladenine (iP), and isopentenyladenosine (iPA) in rice substantially decreased with the decrease in soil moisture but they only slightly increased after rewatering (Bano *et al.* 1993).

Decreased content of CKs and accumulation of abscisic acid (ABA) in water-stressed plants lead to strongly increased ABA/CKs ratio. This ratio was also increased in apoplastic solution of water-stressed cotton and sunflower, but the CK concentration was not significantly changed (Hartung *et al.* 1992, Masia *et al.* 1994). During dehydration of wheat seedlings accumulation of ABA was accompanied by a decline in

the content of zeatin nucleotide and the accumulation of zeatin 9-N-glucoside, but the total content of zeatin derivatives as well as the content of free base of Z remained almost constant (Mustafina *et al.* 1997/98).

After a sudden increase of air temperature from 24 to 35 °C, relative water content (RWC) and ABA content in shoots of wheat seedlings temporarily increased and then gradually declined. The Z content negatively correlated with RWC, it temporarily decreased and then gradually increased. Together with ABA content, Z played an important role in regulation of transpiration rate (Teplova *et al.* 1999).

The concentrations of Z, ZR, iP, and iPA decreased significantly in shoots and roots of barley cultivars after exposure to increased concentration of NaCl (Kuiper *et al.* 1990). In contrast, in pea the CK content increased under salt stress. This increase was mostly due to the strongly elevated riboside levels, especially in roots. A period of 2-d-stress was sufficient to establish significant difference in CK content. During further growth the differences between salt-stressed and control plants disappeared and at flowering stage CK content in stressed plants was near to the controls (Atanassova *et al.* 1999).

## Roles of cytokinins in stomatal regulation of gas exchange

In response to soil moisture depletion stomatal conductance is regulated by hydraulic as well as chemical signals. Their relative importance is likely to depend on plant size and growth conditions (Whitehead 1998). For chemical regulation, the most appropriate candidate is abscisic acid (ABA) and substantial amount of ABA is produced under water stress. The stomata respond to ABA concentration in their vicinity; ABA may be produced directly in the leaves or delivered by xylem from the roots. The precise mechanism is still not known, probably ABA influences capacities of inward or outward rectifying guard cell membrane channels for transport of potassium ions. Also further plant hormones, CK and auxins, can operate in conjunction with ABA.

Both synthetic and naturally occurring CKs increase transpiration rate ( $E$ ) of excised leaves and increase stomatal aperture in isolated epidermes (Incoll and Jewer 1987). The effect is sometimes dependent on the plant age, and it is affected by interactions with the closing effects of  $\text{CO}_2$  and ABA. High concentrations of CKs can override the effects of ABA on stomata; thus reduction in CK supply as soil dries might amplify shoot responses to an increasing concentration of ABA (Davies and Zhang 1991). In desert-grown almond trees CKs showed peak concentrations in the morning and a rapid decrease in the afternoon; these daily variations preceded daily variation in stomatal conductance ( $g_s$ ). The authors conclude that CKs may affect stomatal behaviour on a short-term basis, while ABA concentration reflects long-term water deficit (Fusseder *et al.* 1992).

In parts of maize leaf blade, Z alone did not affect stomatal opening, but it partially reversed ABA-induced stomatal closure. On the contrary, in *Commelina* epidermal strips or leaf fragments, Z or Kin decreased stomatal opening and had no effect on ABA-induced

stomatal closure (Blackman and Davies 1983). In cotton, kinetin (Kin) had little effect on stomata, and on stomatal response to ABA in control plants, but decreased stomatal response to ABA in plants grown under nitrogen deficiency (Radin *et al.* 1982, Radin and Hendrix 1988). In flax, Z alone did not significantly affect  $E$  and net photosynthetic rate ( $P_N$ ), but alleviated the effect of ABA (Drüge and Schönbeck 1992).  $E$  and  $g_s$  of adaxial and abaxial epidermes of leaves of field-grown sugar beet were not significantly affected by foliar spray with synthetic CK  $N^6$ -(*m*-hydroxylbenzyl)adenosine (Čatský *et al.* 1996).  $E$  of tobacco plants grown *in vitro* was increased with increasing concentration of BAP up to  $0.10 \text{ mg dm}^{-3}$  but decreased at higher concentration (Pospíšilová *et al.* 1993). Similarly stomatal aperture of *Digitalis* was greater when grown *in vitro* on medium with Kin or BAP and the effect was concentration dependent (Diettrich *et al.* 1992). In the root hemiparasite *Melampyrum arvense*, application of CKs increased stomatal opening and guard cell  $\text{K}^+$  content only in preparasitic stage, but no effect appeared when attached to the host. From the CKs used, the highest effect was caused by ZR and dihydrozeatin ribosid, followed by dihydrozeatin, Z, iPA, and BAP; iP was almost inactive (Lechowski 1997).

The mechanism of CK action on guard cell might involve direct induction of membrane hyperpolarization by stimulation of electrogenic  $\text{H}^+$ -pump, stimulation of adenylate cyclase activity which could lead to an increase in intracellular adenosine 3',5'-cyclic monophosphate content, stimulation of guanylate cyclase activity, or interaction with calcium-calmodulin system, and with ABA regulation of ion channel permeabilities (Incoll *et al.* 1990, Morsucci *et al.* 1991, Pharmawati *et al.* 1998).

## Effect of cytokinins on other responses of plants to water stress

CKs antagonize many physiological processes induced by water stress, mainly those mediated by ABA. Well known is the above mentioned reversal of ABA-induced stomatal closure. Further, water stress usually accelerates leaf senescence, and on the contrast, CKs delay leaf senescence (*e.g.* Soejima *et al.* 1992, Čatský *et al.* 1996, Naqvi 1999). There is little information available concerning the role of root signals in controlling shoot behaviour during development of plants grown under favourable conditions. CKs may be involved also in this signalling (Meinzer *et al.* 1991). Thus CKs can affect many responses of plants to water stress connected with plant growth, development, and senescence. When naturally occurring CKs were supplied to leaves via

xylem at concentration corresponding approximately to standard concentration of endogenous CKs, they increased transpiration and delayed senescence in oak and wheat (Badenoch-Jones *et al.* 1996). The authors suggested that Z-type bases, ribosides and O-glucosides probably regulated transpiration *in vivo*, O-glucosides might be important regulators of leaf senescence, but nucleotides played a lesser role in controlling these processes.

Application of CKs can reverse leaf and fruit abscission induced by ABA or water stress, or CKs release seed dormancy in contrasts with ABA inhibition of germination. The antagonism between CKs and ABA may be the result of metabolic interactions. CKs share, at

least in part, a common biosynthetic origin with ABA (Cowan *et al.* 1999).

With leaf age, drought induced inhibition of photosynthesis increased and ability to recover after rewatering decreased (*e.g.*, David *et al.* 1998). This fact accentuates the importance of delayed leaf senescence induced by CK. In addition, CK can directly affect photosynthetic parameters, *e.g.*, chlorophyll and photosynthetic protein synthesis and degradation, chloroplast composition and ultrastructure, electron transport, and enzyme activities (for review see Synková *et al.* 1997b).

Application of BAP alleviated negative effect of NaCl on chlorophyll (Chl) content in detached wheat leaves, but not in intact seedlings (Mumtaz *et al.* 1997). BAP and synthetic CK 4-PU-30 increased the photochemical activity in control, water-stressed, and rehydrated bean plants (Metwally *et al.* 1997). In spring wheat, the cytokinin-like compound kartolin-4 alleviated negative effect of water stress on ribulose-1,5-bisphosphate carboxylase content and activity (Chernyad'ev and Monakhova 1998). In *Mesembryanthemum*, application of BAP, Z, or iP induced accumulation of a specific isoform of phosphoenolpyruvate carboxylase and proline. Thus in this plant CKs might promote switch from C<sub>3</sub> to CAM type of carbon metabolism and/or osmotic adjustment similarly as NaCl stress (Thomas *et al.* 1992).

Also in *Cicer*, application of BAP stimulated accumulation of proline, amino acids, and soluble sugars under water stress, *i.e.*, substance which might be responsible for osmotic adjustment (Yadav *et al.* 1997). Stimulation of osmotic adjustment by Kin was found in callus cultures of cowpea (Agarwal and Gupta 1995).

Addition of BAP in concentrations from 0.01 to 1.00 mg dm<sup>-3</sup> did not significantly affect water, osmotic, and pressure potentials of *in vitro* grown tobacco leaves or potato shoots (Pospíšilová *et al.* 1993), but BAP in concentration 0.7 mg dm<sup>-3</sup> caused vitrification of melon plantlets (Leshem *et al.* 1988). Application of BAP to roots or in form of spray to leaves did not significantly speed up regeneration of bean plants after water stress: the effects of 0.001 mM BAP on g<sub>s</sub>, P<sub>N</sub>, and Chl content were slightly positive, those of 0.01 mM BAP slightly negative (Pospíšilová and Rulcová 1999).

In maize, addition of Kin to the bathing medium resulted in a reduction of root exudation (Rhizopoulou and Wagner 1998). In bean plants, application of CK 4-PU-30 alleviated negative effect of water stress on the lipid membrane composition (Ivanova *et al.* 1998). In *Shorea* seeds, Kin delayed desiccation-induced membrane deterioration (Krishna Chaitanya and Naithani 1998).

Plant hormones including CKs regulate expression of a great number of stress-induced genes (for review see, *e.g.*, Itai 1999, Naqvi 1999).

## Water relations of transgenic plants with increased cytokinin content

Recently, the characterization and exploitation of the bacterial CK biosynthesis genes in genetic engineering of higher plants have provided a new tool to the study of regulatory role and mechanisms of CK functioning. Mostly, the *Agrobacterium tumefaciens* Ti-plasmid T-DNA *ipt* gene has been used. This gene codes for isopentenyltransferase, the enzyme which catalyzes the key step in *de novo* CK biosynthesis: the formation of N<sup>6</sup>-(Δ<sup>2</sup>-isopentenyl) adenosine-5'-monophosphate from Δ<sup>2</sup>-isopentenyl pyrophosphate and adenosine-5'-monophosphate. The *ipt* gene has been introduced under its native promoter (Ondřej *et al.* 1989, 1990), promoterless (Hewelt *et al.* 1994), or as chimaeric *ipt* genes with different promoters used to construct the latter ones (Synková *et al.* 1997b). Heat shock, light-inducible, and developmentally controlled promoters have been successfully used (Beinsberger *et al.* 1991, Ainley *et al.* 1993, Gan and Amasino 1995). Resulting CK content and composition was dependent not only on promoter used, but also on plant species and age (Pospíšilová *et al.* 1995, 1997/98, Eklöf *et al.* 1996, Synková *et al.* 1997a). Generally, the *ipt* transgenic plants are characterized by a loss of apical dominance and a release of axillary buds which results in highly branched shoots, and inhibition or

retardation of root formation. Besides, there are many traits the appearance of which depends on the concentration of endogenous CK reached after expression of the *ipt* gene. This indicates that if some threshold concentration is reached or exceeded, the opposite effect to that at lower concentration can be achieved. Thus, an enhanced as well as lowered Chl content, delayed or premature senescence of leaf and plant, different modifications in leaf and stem structures, appearance of viviparous shoots, delay in onset of flowering, retardation of flower development, or partial sterility of seeds were found (for review see Synková *et al.* 1997b).

Moreover, the disturbances in water regime have been observed. While in *ipt*-transgenic tobacco plants with only slightly elevated CK content stomatal and cuticular transpiration rates as well as parameters of water relations were not significantly affected (Čatský *et al.* 1993b, Pospíšilová *et al.* 1997/98), in *ipt* transgenic tobacco, where endogenous CK increased 2 - 10 fold (compared to control untransformed plants) water stress often appeared (Medford *et al.* 1989, Li *et al.* 1992, Hewelt *et al.* 1994, Thomas *et al.* 1995, Synková *et al.* 1995, 1999). However, in potato transgenic plants shoot relative water content, and water, osmotic, and pressure potentials were

not expressively influenced by an increased content of endogenous CKs (Šiffel *et al.* 1992, Čatský *et al.* 1993a). In *zmp*-transgenic tobacco plants (Brzobohatý *et al.* 1993), markedly increased CK content was found only in older leaves and in these leaves  $g_s$  was increased. On the other hand, stomatal conductance of upper leaves was slightly decreased (Pospíšilová *et al.* 1995, 1997/98). In transgenic tobacco plants with heat-inducible expression of *ipt*-gene, heat shock led to elevated content of cytokinins (ten times higher than in control plants), and threefold increase in the transpiration rate (Kudoyarova *et al.* 1999). Wang *et al.* (1997a,b) also reported increased transpiration rates and stomatal conductance associated with increased number and size of stomata in transgenic tobacco with *ipt* gene under control of a chalcone synthase promoter.

In transgenic tobacco with *ipt* gene under control of light inducible promoter of a small unit of Rubisco (*Pssu-ipt*) severe wilting was observed. However, even if CK content was lower in young than in old leaves, significantly lowered relative water content and increased stomatal conductance was found in young leaves in comparison to control, untransformed plants, while in the older ones decreased stomatal conductance was found (Synková *et al.* 1995, 1999). The rate of transpiration exceeding water uptake due to the disproportion of root and shoot systems (high shoot/root ratio) can be the main cause of wilting. Changes in stem thickness caused by an enhancement of both cell division and cell enlargement and increased number of xylem vessels were observed in some *ipt* transgenic tobacco plants (Wang *et al.* 1997a,b, Ainley *et al.* 1993). These changes might partially help to overcome limitations caused by proportionally smaller root system.

## Conclusions

CKs and ABA usually have antagonistic effects. In water relations, the main effects are promotion of stomatal opening and transpiration rate by CKs and inhibition by ABA. The content of endogenous CK mostly decreases under water stress which amplifies the response of shoot to increasing concentration of ABA. Application of natural or synthetic CK might partially ameliorate

Nevertheless, Thomas *et al.* (1995) reported wilting in *Pssu-ipt* tobacco similar to that observed in salt stressed plants. Beside elevated CK content, transgenic plants showed also an enhancement of proline and osmotin concentrations similarly as salt stressed plants. This fact leads authors to conclusion that CKs might elicit responses similar to those induced by environmental stresses including tolerance to subsequent stresses. Stress-like effects were also found in the ultrastructure of chloroplasts in *Pssu-ipt* tobacco (Valcke and Beinsberger 1989, Synková *et al.* 1997a). The swollen thylakoids, accumulation of vesicles and plastoglobuli are the most apparent features. Recent study shows that anomalies involving chloroplast ultrastructure and their relations with other organelles become more numerous with increasing leaf and plant age (Synková, unpublished).

An indisputable effect of elevated content of endogenous CK in transgenic plants is delayed senescence of the leaves and prolonged life span of the whole plant (Synková *et al.* 1997b, Wang *et al.* 1997a,b.). CK induction of developmental changes in transgenic tobacco, essentially an annual plant, converted those plants into polycarpic and potentially perennial species characterized by continuous production of lateral shoots which produced flowers and the developed new shoots directly from the root.

The most promising strategy for distinguishing the role of enhanced stomatal opening and high shoot/root ratio in development of "wilting" phenotype might be to use *ipt* gene under senescence specific promoters (Gan and Amasino 1995). These transgenic plants firstly develop normal root system and high CK production starts at the onset of leaf senescence.

negative effects of water stress, *e.g.*, by stimulation of osmotic adjustment, delay of stress induced senescence, and reversion of leaf and fruit abscission. "Wilting" phenotype of transgenic plants with markedly increased content of endogenous CK may be the result of increased shoot/root ratio and/or decreased stomatal regulation of gas exchange.

## References

- Agarwal, R.K., Gupta, S.C.: Plant growth substances as osmoregulators under salt stress in callus cultures of cowpea. - *Indian J. Plant Physiol.* **38**: 325-327, 1995.
- Ainley, W.M., McNeil, K.J., Hill, J.W., Lingle, W.L., Simpson, R.B., Brenner, M.L., Nagao, R.T., Key, J.L.: Regulatable endogenous production of cytokinins up to toxic levels in transgenic plants and plant tissue. - *Plant mol. Biol.* **22**: 13-23, 1993.
- Atanassova, L.Y., Stoyanov, I.G., Pissarska, M.G.: Salt-induced responses of endogenous cytokinins in pea plants. - *Biol. Plant.* **42** (Suppl.): S73, 1999.
- Badenoch-Jones, J., Parker, C.W., Letham, D.S., Singh, S.: Effect of cytokinins supplied via the xylem at multiples of

- endogenous concentrations on transpiration and senescence in derooted seedlings of oat and wheat. - *Plant Cell Environ.* **19**: 504-516, 1996.
- Bano, A., Dörfling, K., Bettin, D., Hahn, H.: Absciscic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. - *Aust. J. Plant Physiol.* **20**: 109-115, 1993.
- Beinsberger, S.E.I., Valcke, R.L.M., Deblaere, R.Y., Clijsters, H.M.M., De Greef, J.A., Van Onckelen, H. A.: Effects of introduction of *Agrobacterium tumefaciens* T-DNA *ipt* gene in *Nicotiana tabacum* L. cv. Petit Havana SR1 plant cells. - *Plant Cell Physiol.* **32**: 489-496, 1991.
- Blackman, P.G., Davies, W.J.: The effect of cytokinins and ABA on stomatal behaviour of maize and *Commelina*. - *J. exp. Bot.* **34**: 1619-1626, 1983.
- Braut, M., Maldiney, R.: Mechanisms of cytokinin action. - *Plant Physiol. Biochem.* **37**: 403-412, 1999.
- Brzobohatý, B., Moore, I., Kristoffersen, P., Bako, L., Campos, N., Schell, J., Klaus, P.: Release of active cytokinin by a  $\beta$ -glucosidase localized to the maize root meristem. - *Science* **262**: 1051-1054, 1993.
- Čatský, J., Pospíšilová, J., Kamínek, M., Gaudinová, A., Pulkrábek, J., Zahradníček, J.: Seasonal changes in sugar beet photosynthesis as affected by exogenous cytokinin *N*<sup>6</sup>-(*m*-hydroxybenzyl)adenosine. - *Biol. Plant.* **38**: 511-518, 1996.
- Čatský, J., Pospíšilová, J., Macháčková, I., Synková, H., Wilhelmová, N., Šesták, Z.: High level of endogenous cytokinins in transgenic potato plantlets limits photosynthesis. - *Biol. Plant.* **35**: 191-198, 1993a.
- Čatský, J., Pospíšilová, J., Macháčková, I., Wilhelmová, N., Šesták, Z.: Photosynthesis and water relations in transgenic tobacco plants with T-DNA carrying gene 4 for cytokinin synthesis. - *Biol. Plant.* **35**: 393-399, 1993b.
- Chernyad'ev, I.I., Monakhova, O.F.: The activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase in wheat plants as affected by water stress and kartinol-4. - *Photosynthetica* **35**: 603-610, 1998.
- Cowan, A.K., Cairns, A.L.P., Bartels-Rahm, B.: Regulation of absciscic acid metabolism: towards a metabolic basis for absciscic acid-cytokinin antagonism. - *J. exp. Bot.* **50**: 595-603, 1999.
- David, M.M., Coelho, D., Barrote, I., Correia, M.J.: Leaf age effects on photosynthetic activity and sugar accumulation in droughted and rewetted *Lupinus albus* plants. - *Aust. J. Plant Physiol.* **25**: 299-306, 1998.
- Davies, P.J.: The plant hormone concept: concentration, sensitivity and transport. - In: Davies, P.J. (ed.): *Plant Hormones*. Pp. 13-38. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.
- Davies, W.J., Zhang, J.: Root signals and the regulation of growth and development of plants in drying soil. - *Annu. Rev. Plant Physiol. Plant mol. Biol.* **42**: 55-76, 1991.
- Dietrich, B., Mertinat, H., Luckner, M.: Reduction of water loss during *ex vitro* acclimatization of micropropagated *Digitalis lanata* clone plants. - *Biochem. Physiol. Pflanz.* **188**: 23-31, 1992.
- Drüge, U., Schönbeck, F.: Effect of vesicular-arbuscular mycorrhizal infection on transpiration, photosynthesis and growth of flax (*Linum usitatissimum* L.) in relation to cytokinin levels. - *J. Plant Physiol.* **141**: 40-48, 1992.
- Eklöf, S., Åstot, C., Moritz, T., Blackwell, J., Olsson, O., Sandberg, G.: Cytokinin metabolites and gradients in wild type and transgenic tobacco with moderate cytokinin overproduction. - *Physiol. Plant.* **98**: 333-344, 1996.
- Fusseder, A., Waringer, A., Hartung, W., Schulze, E.-D., Heilmeyer, H.: Cytokinins in the xylem sap of desert grown almond (*Prunus dulcis*) trees: Daily courses and their possible interactions with absciscic acid and leaf conductance. - *New Phytol.* **122**: 45-52, 1992.
- Gan, S., Amasino, R.M.: Inhibition of leaf senescence by autoregulated production of cytokinin. - *Science* **270**: 1986-1988, 1995.
- Goicoechea, N., Antolín, M.C., Sánchez-Díaz, M.: Gas exchange is related to the hormonal balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. - *Physiol. Plant.* **100**: 989-997, 1997.
- Goicoechea, N., Doležal, K., Antolín, M.C., Strnad, M., Sánchez-Díaz, M.: Influence of mycorrhizae and *Rhizobium* on cytokin content in drought-stressed alfalfa. - *J. exp. Bot.* **46**: 1543-1549, 1995.
- Hare, P.D., Cress, W.A., Van Staden, J.: The involvement of cytokinins in plant responses to environmental stress. - *Plant Growth Regul.* **23**: 79-103, 1997.
- Hartung, W., Weiler, E.W., Radin, J.W.: Auxin and cytokinins in the apoplastic solution of dehydrated cotton leaves. - *J. Plant Physiol.* **140**: 324-327, 1992.
- Hewelt, A., Prinsen, E., Schell, J., Van Onckelen, H., Schumling, T.: Promoter tagging with promoterless *ipt* gene leads to cytokinin-induced phenotypic variability in transgenic tobacco plants: implications of gene dosage effects. - *Plant J.* **6**: 879-891, 1994.
- Incoll, L.D., Jewer, P.C.: Cytokinins and stomata. - In: Zeiger, E., Farquhar, G.D., Cowan, I.R. (ed.): *Stomatal Function*. Pp. 281-292. Stanford University Press, Stanford 1987.
- Incoll, L.D., Ray, J.P., Jewer, P.C.: Do cytokinins act as root to shoot signals? - In: Davies, W.J., Jeffcoat, B. (ed.): *Importance of Root to Shoot Communication in the Responses to Environmental Stress*. Pp. 185-197. British Society for Plant Growth Regulation, Bristol 1990.
- Itai, C.: Role of phytohormones in plant responses to stresses. - In: Lerner, H.R. (ed.): *Plant Responses to Environmental Stress*. From Phytohormones to Genome Reorganization. Pp. 287-301. Marcel Dekker, New York - Basel 1999.
- Ivanova, A.P., Stefanov, K.L., Yordanov, I.T.: Effect of cytokinin 4-PU-30 on the lipid composition of water stressed bean plants. - *Biol. Plant.* **41**: 155-159, 1998.
- Kamínek, M., Motyka, V., Vaňková, R.: Regulation of cytokinin content in plant cells. - *Physiol. Plant.* **101**: 689-700, 1997.
- Krishna Chaitanya, K.S., Naithani, S.C.: Kinetic-mediated prolongation of viability in recalcitrant sal (*Shorea robusta* Gaertn. f.) seeds at low temperature: role of kinetin in delaying membrane deterioration during desiccation-induced injury. - *J. Plant Growth Regul.* **17**: 63-69, 1998.
- Kudoyarova, G., Valcke, R., Teplova, I., Mustafina, A.: Cytokinin content and transpiration of transgenic tobacco plants containing heat-inducible *ipt*-gene as affected by high temperature. - *Biol. Plant.* **42** (Suppl.): S75, 1999.
- Kuiper, D., Schuit, J., Kuiper, P.J.C.: Actual cytokinin concentrations in plant tissue as an indicator for salt resistance in cereals. - *Plant Soil* **123**: 243-250, 1990.

- Lechowski, Z.: Stomatal response to exogenous cytokinin treatment of the hemiparasite *Melampyrum arvense* L. before and after attachment to the host. - *Biol. Plant.* **39**: 13-21, 1997.
- Leshem, B., Werker, E., Shalev, D.P.: The effect of cytokinins on vitrification in melon and carnation. - *Ann. Bot.* **62**: 271-276, 1988.
- Levine, A.: Oxidative stress as a regulator of environmental responses in plants. - In: Lerner, H.R. (ed.): *Plant Responses to Environmental Stresses. From Phytohormones to Genome Organization*. Pp. 247-264. Marcel Dekker, New York - Basel 1999.
- Li, Y., Hagen, G., Guilfoyle, T.J.: Altered morphology in transgenic tobacco plants that overproduce cytokinins in specific tissues and organs. - *Developmental Biol.* **153**: 386-395, 1992.
- Masia, A., Pitacco, A., Braggio, L., Giulivo, C.: Hormonal responses to partial drying of the root system of *Helianthus annuus*. - *J. exp. Bot.* **45**: 69-76, 1994.
- Medford, J.I., Horgan, R., El-Sawi, Z., Klee, H.J.: Alteration of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. - *Plant Cell* **1**: 403-413, 1989.
- Meinzer, F.C., Grantz, D.A., Smit, B.: Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. - *Aust. J. Plant Physiol.* **18**: 329-338, 1991.
- Metwally, A., Tsonev, T., Zeinalov, Y.: Effect of cytokinins on the photosynthetic apparatus in water-stressed and rehydrated bean plants. - *Photosynthetica* **34**: 563-567, 1997.
- Morsucci, R., Curvetto, N., Delmastro, S.: Involvement of cytokinins and adenosine 3',5'-cyclic monophosphate in stomatal movement in *Vicia faba*. - *Plant Physiol. Biochem.* **29**: 537-547, 1991.
- Mumtaz, S., Naqvi, S.S.M., Shereen, A., Khan, M.A.: Salinity stress and the senescence process in wheat (*Triticum aestivum* L.). - *Pakistan J. Bot.* **29**: 299-303, 1997.
- Mustafina, A., Veselov, S., Valcke, R., Kudoyarova, G.: Contents of abscisic acid and cytokinins in shoots during dehydration of wheat seedlings. - *Biol. Plant.* **40**: 291-293, 1997/98.
- Naqvi, S.S.M.: Plant hormones and stress phenomena. - In: Pessarakli, M. (ed.): *Handbook of Plant and Crop Stress*. Pp. 383-400. Marcel Dekker, New York - Basel - Hong Kong 1994.
- Naqvi, S.S.M.: Plant/crop hormones under stressful conditions. - In: Pessarakli, M. (ed.): *Handbook of Plant and Crop Physiology*. Pp. 645-660. Marcel Dekker, New York - Basel - Hong Kong 1995.
- Naqvi, S.S.M.: Plant hormones and stress phenomena. - In: Pessarakli, M. (ed.): *Handbook of Plant and Crop Stress*. Pp. 709-730. Marcel Dekker, New York - Basel 1999.
- Ondřej, M., Hrouda, M., Karavajko, N.N., Matoušek, J., Mikulovič, T.P., Pavingerová, D., Vlasák, J.: Transformation by *Agrobacterium* vectors and the study of function of plant hormones. - In: Krekule, J., Seidlová, F. (ed.): *Signals in Plant Development*. Pp. 73-89. SPB Academic Publishing, The Hague 1989.
- Ondřej, M., Macháček, I., Čatský, J., Eder, J., Hrouda, M., Pospíšilová, J., Synková, H.: Potato transformation by T-DNA cytokinin synthesis gene. - *Biol. Plant.* **32**: 401-406, 1990.
- Pharmawati, M., Billington, T., Gehring, C.A.: Stomatal guard cell responses to kinetin and natriuretic peptides are cGMP-dependent. - *Cell. mol. Life Sci.* **54**: 272-276, 1998.
- Pospíšilová, J., Čatský, J., Synková, H., Macháček, I., Solárová, J.: Gas exchange and *in vivo* chlorophyll fluorescence in potato and tobacco plantlets *in vitro* as affected by various concentrations of 6-benzylaminopurine. - *Photosynthetica* **29**: 1-12, 1993.
- Pospíšilová, J., Rulcová, J.: Can synthetic cytokinins alleviate water stress in *Phaseolus vulgaris* leaves? - *Biol. Plant.* **42** (Suppl.): S77, 1999.
- Pospíšilová, J., Synková, H., Macháček, I., Čatský, J.: Photosynthesis in transgenic tobacco plants. - In: Mathis, P. (ed.): *Photosynthesis: from Light to Biosphere*. Vol. V. Pp. 519-522. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.
- Pospíšilová, J., Synková, H., Macháček, I., Čatský, J.: Photosynthesis in different types of transgenic tobacco plants with elevated cytokinin content. - *Biol. Plant.* **40**: 81-89, 1997/98.
- Radin, J.W., Hendrix, D.L.: The apoplastic pool of abscisic acid in cotton leaves in relation to stomatal closure. - *Planta* **174**: 180-186, 1988.
- Radin, J.W., Parker, L.L., Guinn, G.: Water relation of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. - *Plant Physiol.* **70**: 1066-1070, 1982.
- Rhizopoulou, S., Wagner, E.: Probing short-term root exudation in *Zea mays*. - *Environ. exp. Bot.* **40**: 229-235, 1998.
- Šiffel, P., Šindelková, E., Dürchan, M., Zajícová, M.: Photosynthetic characteristics of *Solanum tuberosum* L. plants transformed by *Agrobacterium* strains. I. Pigment apparatus. - *Photosynthetica* **27**: 441-447, 1992.
- Soejima, H., Sugiyama, T., Ishihara, K.: Changes in cytokinin activities and mass spectrometric analysis of cytokinins in root exudates of rice plant (*Oryza sativa* L.). Comparison between cultivars Nipponbare and Akenohoshi. - *Plant Physiol.* **100**: 1724-1729, 1992.
- Synková, H., Pospíšilová, J., Valcke, R.: Photosynthesis in transgenic *pssu-ipt* tobacco plants as affected by water stress. - In: Mathis, P. (ed.): *Photosynthesis: from Light to Biosphere*. Vol. IV. Pp. 561-564. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.
- Synková, H., Van Loven, K., Pospíšilová, J., Valcke, R.: Photosynthesis of transgenic *Pssu-ipt* tobacco. - *J. Plant Physiol.* **155**: 173-182, 1999.
- Synková, H., Van Loven, K., Valcke, R.: Increased content of endogenous cytokinins does not delay degradation of photosynthetic apparatus in tobacco. - *Photosynthetica* **33**: 595-608, 1997a.
- Synková, H., Wilhelmová, N., Šesták, Z., Pospíšilová, J.: Photosynthesis in transgenic plants with elevated cytokinin contents. - In: Pessarakli, M. (ed.): *Handbook of Photosynthesis*. Pp. 541-552. Marcel Dekker, New York - Basel - Hong Kong 1997b.
- Tardieu, F., Davies, W.J.: Root-shoot communication and whole-plant regulation of water flux. - In: Smith, J.A.C., Griffiths, H.: *Water Deficits. Plant Responses from Cell to Community*. Pp. 147-162. Bios Scientific Publishers, Oxford 1993.

- Teplova, I., Farkhutdinov, R., Mitrichenko, A., Kudoyarova, G.: Changes in zeatin and ABA content and water relations in wheat seedlings as influenced by elevated temperature. - Biol. Plant. **42** (Suppl.): S78, 1999.
- Thomas, J.C., McElwain, E.F., Bohnert, H.J.: Convergent induction of osmotic stress-response. Absciscic acid, cytokinin, and the effects of NaCl. - Plant Physiol. **100**: 416-423, 1992.
- Thomas, J.C., Smigocki, A.C., Bohnert, H.J.: Light-induced expression of *ipt* from *Agrobacterium tumefaciens* results in cytokinin accumulation and osmotic stress symptoms in transgenic tobacco. - Plant mol. Biol. **27**: 225-235, 1995.
- Valcke, R., Beinsberger, S.: Cellular ultrastructure of T-DNA-IPT transformed calli. - Micron microscop. Acta **20**: 135-136, 1989.
- Wang, J., Letham, D.S., Cornish, E., Stevenson, K.R.: Studies of cytokinin action and metabolism using tobacco plants expressing either the *ipt* or the *GUS* gene controlled by a chalcone synthase promoter. I. Developmental features of the transgenic plants. - Aust. J. Plant Physiol. **24**: 661-672, 1997a.
- Wang, J., Letham, D.S., Cornish, E., Wei, K., Hocart, C.H., Michael, M., Stevenson, K.R.: Studies of cytokinin action and metabolism using tobacco plants expressing either the *ipt* or the *GUS* gene controlled by a chalcone synthase promoter. II. *ipt* and *GUS* gene expression, cytokinin levels and metabolism. - Aust. J. Plant Physiol. **24**: 673-683, 1997b.
- Whitehead, D.: Regulation of stomatal conductance and transpiration in forest canopies. - Tree Physiol. **18**: 633-644, 1998.
- Yadav, N., Gupta, V., Yadav, V.K.: Role of benzyladenine and gibberellic acid in alleviating water-stress effect in gram (*Cicer arietinum*). - Indian J. agr. Sci. **67**: 381-387, 1997.