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A CYTOKININ POTION IN PLANT LIVES:
ALMOST AS MIRACULOUS AS LIGHT

Doctoral Thesis

by
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Declaration I

Hereby I declare that the Ph.D. thesis is my original work that I worked up by myself by using the literature which is presented in the section “References”.

In Olomouc, 28 May 2010

Alexandra Husičková

List of papers

The thesis is based on the following papers. These papers are referred in the text by the corresponding roman numerals and are enclosed in the Supplement.

- I. **Vlčková, A.**, Špundová, M., Kotabová, E., Novotný, R., Doležal, K. & Nauš, J. (2006): Protective cytokinin action switches to damaging during senescence of detached wheat leaves in continuous light. *Physiologia Plantarum* 126, 257-267.
- II. Prokopová, J., Špundová, M., Sedlářová, M., **Husičková, A.**, Novotný, R., Doležal, K., Nauš, J. & Lebeda, A. (2010): Photosynthetic responses of lettuce to downy mildew infection and cytokinin treatment. *Plant Physiology and Biochemistry*, in press, doi: 10.1016/j.plaphy.2010.04.003
- III. Hendrickson, L. ^{*}, **Vlčková, A.** ^{*}, Selstam, E., Huner, N., Öquist, G. & Hurry, V. (2006): Cold acclimation of the *Arabidopsis* *dgd1* mutant results in recovery from photosystem I-limited photosynthesis. *FEBS Letters* 580, 4959-4968.

^{*} These authors contributed equally to this manuscript.

Declaration II

I declare that my role in preparation of these papers was as following:

- I. Chief author – project of experiments, most of measurements and experimental procedures, preparation of the manuscript
- II. Co-author – collaboration on measurements
- III. One of two chief authors – all measurements and experimental procedures except the gas exchange analysis, collaboration on preparation of the manuscript

Alexandra Husičková.....

On behalf of the co-authors, the declaration II was confirmed by:

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Unfortunately I did not reach my foreign colleagues to get a confirmation concerning the paper III; however, my contribution to this manuscript was inscribed in the paper by itself by the statement indicated above as ^{*}.

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Research fellowship

01/2005 – 06/2005 Umeå Plant Science Centre and Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology; Supervisor: Dr. Vaughan Hurry

Publications

1. Špundová, M., **Vlčková, A.**, Doležal, K., Habertová, A., Nauš, J. & Strnad, M. (2001): Effect of *meta*-topolin and boheminine derived from benzylaminopurine on PSII function in artificially senescing wheat leaves. In PS2001 Proceedings of the 12th International Congress on Photosynthesis S22-012, CSIRO Publishing, Collingwood, Australia,
2. Špundová, M., Prokopová, J., Sedlářová, M., Výtisková, M. & **Vlčková, A.** (2005): Effect of exogenous application of cytokinins on pathogenesis of *Bremia lactucae* and photosynthetic parameters of host plant. In Proceedings of the conference “The effect of abiotic and biotic stressors on plant characteristics”, Prague, Czech Republic, 302-308.
3. **Vlčková, A.**, Špundová, M., Kotabová, E., Novotný, R., Doležal, K. & Nauš, J. (2006): Protective cytokinin action switches to damaging during senescence of detached wheat leaves in continuous light. *Physiologia Plantarum* 126, 257-267.
4. Hendrickson, L., **Vlčková, A.**, Selstam, E., Huner, N., Öquist, G. & Hurry, V. (2006): Cold acclimation of the *Arabidopsis dgdl* mutant results in recovery from photosystem I-limited photosynthesis. *FEBS Letters* 580, 4959-4968.
5. Selstam, E., Hendrickson, L., Krol, M., Ivanov, A.G., **Vlčková, A.**, Huner, N., Öquist, G. & Hurry, V. (2006) Low temperature recovery of photosynthesis in DGDG deficient mutant of *Arabidopsis*. In: *Photosynthesis in the Post-Genomic Era: Structure and Function of Photosystems – Conference Proceeding*, Pushchino, Russia.
6. Prokopová, J., Špundová, M., Sedlářová, M., **Husičková, A.**, Novotný, R., Doležal, K., Nauš, J. & Lebeda, A. (2010): Photosynthetic responses of lettuce to downy mildew infection and cytokinin treatment. *Plant Physiology and Biochemistry*, in press, doi: 10.1016/j.plaphy.2010.04.003

Conference presentations

Kotabová, E., **Vlčková, A.**, Novák, O., Špundová, M. & Ilík, P. (2004): The effect of *meta*-topolin on photosynthetic pigment composition in wheat leaves during artificial senescence on continuous light or in the dark. (14th FESPB Congress, Warszawa, Poland) *Acta Physiologiae Plantarum* 26, 102.

Vlčková, A., Kotabová, E., Ilík, P., Novotný, R., Lípová, L. & Špundová, M. (2005): Cytokinin *meta*-topolin induced modification of photosynthetic apparatus in senescing wheat leaves. (15th IUAP and 5th EBSA International Biophysics Congress, France, Montpellier) *European Biophysics Journal with Biophysics Letters* 34, 814.

Vlčková, A., Hendrickson, L., Selstam, E., & Hurry, V. (2005): Impaired photosynthetic apparatus of *Arabidopsis* mutant *dgdl* recovers during cold acclimation. In: *The XXII Congress of the Scandinavian Plant Physiology Society – Book of Abstracts*, Umeå, Sweden.

Ivanov, A.G., Hendrickson, L., Krol, M., Selstam, E., **Vlčková, A.**, Huner, N.P.A., Öquist, G. & Hurry, V. (2005) Digalactosyl-diacylglycerol deficiency alters PSI assembly and function in *Arabidopsis*. In: The XXII Congress of the Scandinavian Plant Physiology Society – Book of Abstracts, Umeå, Sweden.

Špundová, M., Prokopová, J., Sedlářová, M., Výtisková, M. & **Vlčková, A.** (2005): Effect of exogenous application of cytokinins on pathogenesis of *Bremia lactucae* and photosynthetic parameters of host plant. In Proceedings of the conference “The effect of abiotic and biotic stressors on plant characteristics”, Prague, Czech Republic, 302-308.

Sedlářová, M., Výtisková, M., Doležal, K., Špundová, M., **Vlčková, A.**, Prokopová, J. & Lebeda, A. (2005) Treatment with cytokinins delays chlorophyll degradation induced by *Bremia lactucae* pathogenesis on host *Lactuca* spp. Conference “Auxins and Cytokinins in Plant Development”, Prague, Czech Republic.

Selstam, E., Hendrickson, L., Krol, M., Ivanov, A.G., **Vlčková, A.**, Huner, N., Öquist, G. & Hurry, V. (2006) Low temperature recovery of photosynthesis in DGDG deficient mutant of *Arabidopsis*. In: Photosynthesis in the Post-Genomic Era: Structure and Function of Photosystems, Pushchino, Russia.

Citations

Hoelzl, G. & Doermann, P. (2007) Structure and function of glycolipids in plants and bacteria. *Progress in Lipid Research* **46**, 225-243. (cited paper No. 4)

Kotabová, E., Kaňa, R., Kyseláková, H., Lípová, L., Novák, O. & Ilík, P. (2008) A pronounced light-induced zeaxanthin formation accompanied by an unusually slight increase in non-photochemical quenching: A study with barley leaves treated with methyl viologen at moderate light. *Journal of Plant Physiology* **165**, 1563-1571. (cited paper No. 3)

Ruelland, E., Vaultier, M.N., Zachowski, A. & Hurry, V. (2009) Cold Signalling and Cold Acclimation in Plants. In: *Advances in Botanical Research*, **49**, 35-150. (cited paper No. 4)

Torres-Franklin, M.L., Gigon, A., de Melo, D.F., Zuily-Fodil, Y. & Pham-Thi, A.T. (2007) Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. *Physiologia Plantarum* **131**, 201-210. (cited paper No. 4)

Zavaleta-Mancera, H.A., Lopez-Delgado, H., Loza-Tavera, H., Mora-Herrera, M., Trevilla-Garcia, C., Vargas-Suarez, M. & Ougham, H. (2007) Cytokinin promotes catalase and ascorbate peroxidase activities and preserves the chloroplast integrity during dark-senescence. *Journal of Plant Physiology* **164**, 1572-1582. (cited paper No. 3)

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Abbreviations

ABA	abscisic acid
AHK	<i>Arabidopsis</i> histidine-kinase (transmembrane cytokinin receptor)
AHP	<i>Arabidopsis</i> histidine-containing phosphotransfer protein
ARR	<i>Arabidopsis</i> response regulator
Asp	asparagine
BA	N ⁶ -benzyladenine
CAB	gene for the light-harvesting chlorophyll <i>a/b</i> proteins of PSII
COP1	E3 ubiquitin ligase, a negative regulator of photomorphogenesis (constitutively photomorphogenic 1)
CKX	cytokinin oxidase/dehydrogenase; EC 1.4.3.18/1.5.99.12
CRY	cryptochrome
E	transpiration rate
ETR _{max}	maximum electron transport rate
F _v /F _m	maximal photochemical efficiency of photosystem II
g _s	stomatal conductance
His	histidine
HY5	transcription factor positively regulating photomorphogenesis (elongated hypocotyl 5)
iP	N ⁶ -(Δ ² -isopentenyl)adenine
iPR	N ⁶ -(Δ ² -isopentenyl)adenine riboside
<i>ipt</i>	gene for isopentenyltransferase, an enzyme of cytokinin biosynthesis
LHCII	light harvesting chlorophyll protein complex of photosystem II
mT	<i>meta</i> -topolin (N ⁶ -(<i>meta</i> -hydroxybenzyl)adenine)
PCD	programmed cell death
Pfr	far-red-light-absorbing form of phytochrome
phyA	phytochrome A
phyB	phytochrome B
P _N	net photosynthetic rate
Pr	red-light-absorbing form of phytochrome
PSI	photosystem I
PSII	photosystem II
q _p	photochemical chlorophyll fluorescence quenching
<i>rbcS</i>	gene for small subunit of Rubisco
Rfd	ratio of fluorescence decrease (vitality index)
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39
TZD	thidiazuron

tZR	<i>trans</i> -zeatin 9-riboside
Φ_{PSII}	actual quantum yield of photosystem II electron transport
$1-q_P$	excitation pressure on photosystem II
4-PU-30	N-phenyl-N'-(2-chloro-4-pyridyl)urea

Abstract

Similarly as in the animal kingdom, in the floral realm all development and growth are governed by hormones. One of the best and longest known groups of plant hormones bears the name cytokinins. Cytokinins are documented to take part in many developmental processes, including the senescence (“ageing” leading to death of particular plant parts) and handling of adverse environmental conditions. Although these plant hormones are well known for their ability to retard or slow down senescence, there are pieces of evidence that cytokinins can induce acceleration of senescence. Going through the literature, we have noticed that this reversal of cytokinin influence is connected with either their high concentration or exposition of leaves to high light doses, or both. On the basis of this assumption, substantiated by many reports, a new model for cytokinin action is presented here. Many of plant responses to cytokinin treatment imitate responses to light, and plants (plant parts) experiencing both cytokinin and light treatment simultaneously often exhibit the sum of the effects of these signals. However, when the sum of the light and cytokinin influence reaches a certain threshold the effect of their action turns over. In virtue of our observations a possible mechanism of the reversal of cytokinin action during artificial senescence is proposed. This mechanism, nevertheless, cannot explain many other responses originating in light and cytokinin interaction. Looking for a more general connection of cytokinin and light action the literature concerning cytokinin and light signalling pathways was researched. A brief review of common intermediates in cytokinin and light signalling is presented at the end of third chapter of this dissertation. The next part is dedicated to the influence of cytokinins on plants dealing with stressful conditions. Water deficit, being one of the most frequent plants stresses, comprises the best documented stress in the connection with cytokinin influence, thus it deserved a short section in my thesis. Further stress, pathogenic (mildew) infection, was the other aim of our interest and research. Some reports indicate that cytokinins could serve as a disease control agent; our results, however, contradicted the potential utility of cytokinins sprays in field conditions, at least for lettuce endangered by downy mildew. My further research should have been concerned with the influence of cytokinin application on plants undergoing cold stress, which is supposedly the least documented stress in the connection with cytokinin treatment. Nevertheless, our experiments (at the beginning intended to be pre-experiments) concerning the *dgd1 Arabidopsis* mutant during cold acclimation brought such interesting results, that it gained a separate manuscript. Hence, the “cytokinin work” remained for future research. My dissertation is based on three publications that are dealing with different aspects of plant physiology. Taking this fact into consideration, rather than rewriting the results and discussions of my research that have already been published and are enclosed, I decided to compile my doctoral thesis as a mini-review. A brief overview of the original results obtained during my Ph.D. research is presented in the “Summary”.

1 Introduction

Cytokinins constitute a class of plant hormones that were first identified as factors promoting cell division during the 1950s and 1960s (Miller *et al.* 1955; Letham 1963). Since then the spectrum of cytokinin action has been largely extended. Today, it is evident that cytokinins, N⁶-substituted adenine derivatives, are involved in the control of numerous important processes associated with plant growth and development. They take part in the control of cell division, chloroplast development, bud and root differentiation, shoot meristem initiation and growth, stress tolerance, and senescence (Mok & Mok 2001; Schmölling 2002; Choi & Hwang 2007; Werner & Schmölling 2009; Perilli *et al.* 2010). At the cell level, cytokinins act by controlling the expression of many genes (Schmölling *et al.* 1997) and by stimulating the chloroplast development (Synková *et al.* 1997b; Chernyad'ev 2000). Cytokinins increase also the rate of photosynthetic assimilation of carbon dioxide and activities of carbon metabolism enzymes: ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) (Lerbs *et al.* 1984; Kusnetsov *et al.* 1994; Chernyad'ev 1995); NAD-malate dehydrogenase (EC 1.1.1.37); and NADP-glyceraldehydephosphate dehydrogenase complex (EC 1.2.1.13 and EC 4.1.1.31) (Chernyad'ev 2002).

Cytokinin structural diversity and its relation to the biological activity, together with cytokinin biosynthesis, metabolism and translocation are well summarized in reviews of e.g. Sakakibara (2006), Hirose *et al.* (2008), Ma (2008) or Kamada-Nobusada and Sakakibara (2009). The naturally occurring cytokinins have been divided into five functionally different groups: (1) physiologically active cytokinins [*trans*-zeatin (tZ), dihydrozeatin (DHZ) and N⁶- Δ^2 -isopentenyl)adenine (iP) (Figure 1) and other] and their ribosides [tZ 9-riboside (tZR), DHZR, iPR]; (2) transport and storage forms represented by cytokinin O-glucosides, which can be hydrolysed to active forms [tZ O-glucoside (tZOG), tZROG, DHZROG]; (3) cytokinin nucleotides which are believed to be primary, physiologically inactive, products of cytokinin biosynthesis and precursors of their active forms [tZR-5'-monophosphate (tZRMP), DHZRM, iPRM,]; (4) physiologically inactive cytokinin 7- and 9-glucosides which cannot be converted back into the active forms; and (5) *cis*-zeatin and its derivatives (*cis*-zeatin, *cis*-zeatin 9-riboside and *cis*-zeatin 9-riboside-5'-monophosphate) (for review see Zažímalová *et al.* 1999). In figure 1 are displayed also synthetic cytokinins with high physiological activity which are most often used for studies of exogenous application of this hormone.

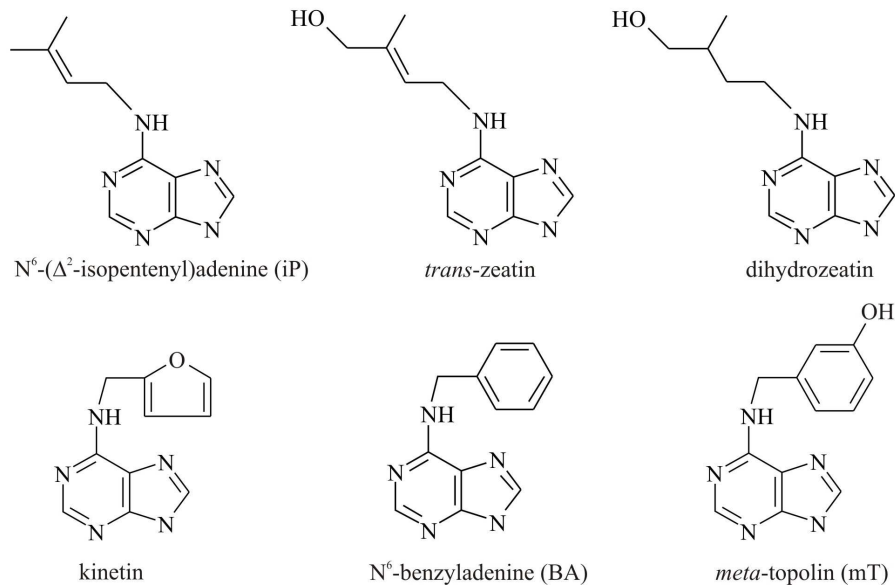


Figure 1. Structures of physiologically active cytokinins that are naturally abundant and/or most often used for exogenous application.

4 Stress & cytokinins

Plants very frequently confront biotic or abiotic stress environment and their sessile style of existence requires that they must show a considerable capacity to respond to these hardships. Morphological and physiological adaptations to stress conditions are very important for plant growth and development and therefore play significant roles in agriculture. It is known that under adverse environmental conditions endogenous cytokinin levels tend to decrease. Possible roles for cytokinins in affecting the overall hormonal balance following environmental stresses, and thereby their role in systemic response to stress, were discussed in Hare *et al.* (1997). Argueso *et al.* (2009) highlighted the most recent advances in the understanding of cytokinin signalling and their interaction with various environmental signals modulating plant growth, development and physiology. The protective action of cytokinins on the photosynthetic machinery and plant productivity under stress was reviewed by Chernyad'ev (2009).

4.1 Drought stress

The availability of water strongly restricts terrestrial plant production. A request for an increase of agriculture production brought demands on researchers to understand controls over plant water relations and consequences of drought stress. In drought stressed plants, cytokinin synthesis in the roots and delivery to leaves is usually decreased (for review see Pospíšilová 2003b). Water deficit, being one of the by plant most often suffered stresses, belongs to the best documented abiotic stresses in connection with cytokinin influence.

Drought induces a number of responses in plants including leaf senescence which plays a major role in the survival of several species (Munné-Bosch & Alegre 2004). Drought-induced leaf senescence contributes to nutrient remobilisation, thus allowing the rest of the plant (i.e. the youngest leaves, fruits or flowers) to benefit from the nutrients accumulated during the life span of older leaves. In addition, drought-induced leaf senescence avoids large water losses through transpiration, thereby contributing to the maintenance of a favourable water balance of the whole plant. However, this drought-accelerated senescence leads to a decrease in canopy size, loss in photosynthesis and reduced yields, and in this way limits productivity of agricultural crops worldwide. Water losses through transpiration and consequent shoot desiccation are also avoided by stomatal regulation (Pospíšilová *et al.* 2000). Due to markedly decreased transpiration rate (E) water content in the leaf during drought stress can remain sufficiently high to prevent a real damage of photosynthetic apparatus.

Cytokinins (applied either exogenously or elevated endogenously via expression of transgenic *ipt* gene) have been shown to alleviate drought-induced leaf senescence (Clark *et al.* 2004; Munné-Bosch & Alegre 2004) as well as the stomatal reduction of transpiration (Todorov *et al.* 1998; Kumar *et al.* 2001; Rulcová & Pospíšilová 2001; She & Song 2006). The question arises as to whether the cytokinin action attenuating these plant defence reactions will heighten plant productivity or consequences of the water deficit.

Exogenous application of cytokinin scaled down the drought stress induced changes in chlorophyll and carotenoid contents (Todorov *et al.* 1998; Rulcová & Pospíšilová 2001; Chernyad'ev & Monakhova 2003; Haisel *et al.* 2006), net photosynthetic rate (P_N) (Kumar *et al.* 2001; Rulcová & Pospíšilová 2001; Pospíšilová 2003a), photochemical (Hill reaction) activity, number of oxygen evolving centres (both the latter Metwally *et al.* 1997), and chlorophyll fluorescence parameters reflecting the function of photosynthetic apparatus: vitality index (Rfd) (Haisel *et al.* 2006), maximal photochemical efficiency of photosystem II (F_v/F_m) (Rulcová & Pospíšilová 2001; Haisel *et al.* 2006), and photochemical chlorophyll fluorescence quenching (q_p) (Yordanov *et al.* 1999; Haisel *et al.* 2006). Furthermore, cytokinin protected chloroplast ultrastructure under drought stress (Stoyanova & Yordanov 1999), alleviated negative effects of water deficit on lipid membrane composition (Ivanova *et al.* 1998), soluble protein content (Todorov *et al.* 1998; Chernyad'ev & Monakhova 2003), catalase activity, H_2O_2 accumulation, and lipid peroxidation (Todorov *et al.* 1998). In addition, Chernyad'ev and Monakhova demonstrated that cytokinins and cytokinin-like substances reduced the dehydration-induced inhibition of activity of some photosynthetic enzymes, namely: Rubisco, NADP: glyceraldehyde-phosphate dehydrogenase complex (determines the carbohydrate route of photosynthetic metabolism at the initial stages), NAD malate dehydrogenase and phosphoenolpyruvate carboxylase (Chernyad'ev 1995; Chernyad'ev & Monakhova 1998, 2001, 2003, 2006). The issue of plant photosynthesis under drought stress and cytokinin protective effects Chernyad'ev discussed in more detail in his review (Chernyad'ev 2005).

Cytokinins favourably affected also recovery of plants during their rehydration (Yordanov *et al.* 1997; Pospíšilová 2003a; Vomáčka & Pospíšilová 2003). Thus the question whether the cytokinin action heightens plant productivity or amplify consequences of the water deficit may seem to be answered; however, there exist evidences that cytokinin application had no effect or even deteriorated the impact of drought stress. For instance, cytokinin effects on stomatal opening, E and P_N are strongly dependent on plant species, cytokinin concentration, way of application and duration of cytokinin action (Pospíšilová *et*

al. 2001; Rulcová & Pospíšilová 2001; Vomáčka & Pospíšilová 2003; Pospíšilová & Bařková 2004). Accordingly, we can see again that action of cytokinins cannot be generalized as also here cytokinins do not act in isolation but other phytohormones participate as well (Pospíšilová 2003b). Involvement in the regulation of stomatal opening has been generally accepted foremost for the abscisic acid (ABA). ABA influences not only the endogenous cytokinin content during drought stress but also the action of exogenously applied cytokinins (Pospíšilová 2003a; Pospíšilová *et al.* 2005). Additionally, an evidence exists that cytokinin action during drought stress is tightly connected also with nitric oxide signal (Shao *et al.* 2010).

With promising results for agriculturalists, which support the idea of genetically modified plants, came Rivero and her colleagues. They generated transgenic tobacco plants expressing an isopentenyltransferase gene (*ipt* gene coding an enzyme of cytokinin biosynthesis) driven by a maturation- and drought stress- induced promoter SARK (senescence associated protein kinase) (Rivero *et al.* 2007). The SARK-*ipt* expression reached maxima levels in all leaves of the transgenic plants during the drought stress, accompanied with a significant increase in *trans*-zeatin levels. This increase in cytokinin contents resulted in suppression of drought-induced leaf senescence and in an outstanding drought tolerance. The $P_{SARK}::IPT$ transgenic tobacco exhibited vigorous growth after a two week drought period that killed the control plants (Figure 14). Moreover, the transgenic plants displayed minimal yield loss when watered with only 30% of the amount of water used under normal conditions (Rivero *et al.* 2007). Further investigation of Rivero *et al.* (2009) revealed that cytokinins during the growth under water limitation mediated induction of photorespiration. Thus cytokinins play a considerable role also in the protection of biochemical processes associated with photosynthesis under water deficit (Rivero *et al.* 2009).

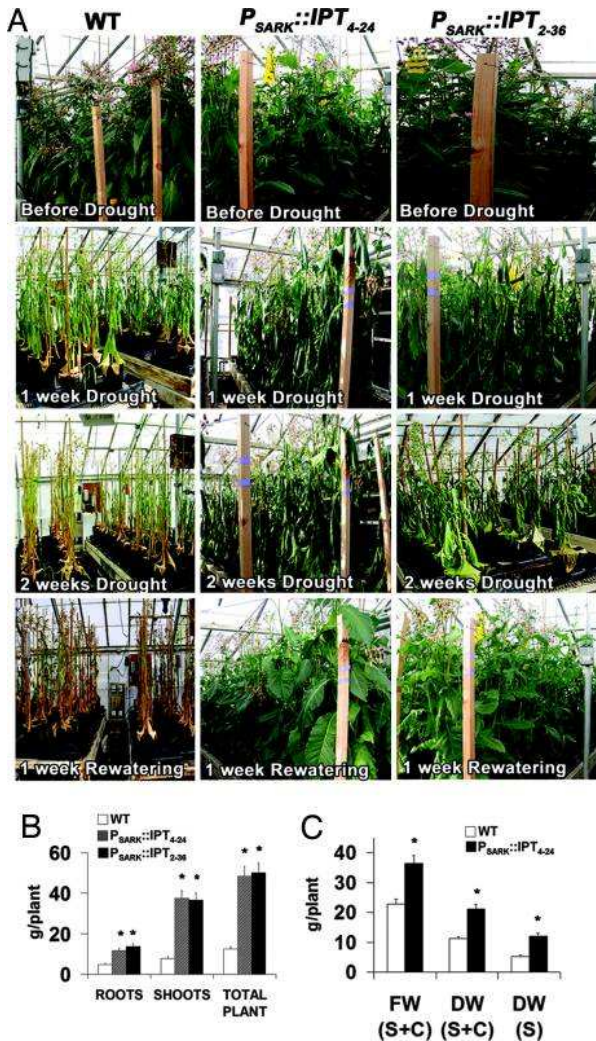


Figure 14. Drought effects on *Arabidopsis* WT and two lines of $P_{SARK::IPT}$ transgenic tobacco plants ($P_{SARK::IPT_{2-36}}$ and $P_{SARK::IPT_{4-24}}$) (with permission⁴ reprinted from Rivero et al. 2007). (A) Plants before drought, after 1 and 2 weeks of drought, and after 1 week of rewatering. (B) Dry weight (DW) of the plants (root, shoot, and whole plant) after drought/rewatering experiments. (C) Fresh weight (FW) and dry weight (DW) of seeds (S) and capsules containing seeds (C) collected at the end of the drought/rewatering experiments. Values are mean \pm SE ($n = 40$). * indicate significant differences ($P < 0.001$) between WT and transgenic lines.

⁴ Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. & Blumwald, E. (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19631-19636. Copyright (2007) National Academy of Sciences, U.S.A.

4.2 Pathogenic infection

Disease invasions constitute a major biotic stress to plant, and cytokinins are involved in mediating developmental changes caused by interactions with the pathogen. Both changes in endogenous cytokinin contents and exogenous applications of cytokinins affect plant interactions with pathogens (Ashby 2000; Talieva *et al.* 2001; Yarullina *et al.* 2001; Walters & McRoberts 2006; Bari & Jones 2009). Applying cytokinins to diseased plants has been known to have positive effects, including both delayed plant senescence and arrest of pathogen development, for several decades. For instance, relationships between effects of kinetin, concentrations of applied solutions and fungal developmental stages in wheat-*Fusarium culmorum* interactions were reported in the 1980s (Michniewicz *et al.* 1984). Kinetin has also been shown to diminish the number and size of necrotic lesions caused by the necrotrophic fungus *Bipolaris sorokiniana* on barley (Sarhan *et al.* 1991), and Mills *et al.* (1986) found that BA pre-treatment reduced the size of lesions on cucumber leaves infected by the hemibiotrophic fungus *Colletotrichum lagenarium*. However, some authors have found more ambiguous results for biotrophic powdery mildews. For example, Mishina *et al.* (2002) observed increases in the germination rate and formation of abnormal appressoria of *Golovinomyces cichoracearum* following exposure to *trans*-zeatin and kinetin. Further, application of these two cytokinins caused reduction in the intensity of conidia germination on infected phlox leaves, but had no significant effect on infected barley. Even though application of exogenous cytokinins may lead to suppression of pathogen development, or enhancement of plant resistance (Sarhan *et al.* 1991; Mishina *et al.* 2002); strikingly, the level and activity of plants' endogenous cytokinins may be increased due to the infection process (Talieva *et al.* 2001; Yarullina *et al.* 2001). Transcriptome analysis of clubroots of *Arabidopsis* plants inoculated by *Plasmodiophora brassicae* showed that genes associated with cytokinin homeostasis (cytokinin synthase *IPT3*; cytokinin oxidase/dehydrogenases *CKX1* and *CKX6*) are strongly down-regulated early in pathogen colonization (Siemens *et al.* 2006). Expression of many genes encoding proteins involved in cytokinin signalling was markedly influenced as well, in comparison to not inoculated *Arabidopsis* plants. Moreover transgenic plants overexpressing cytokinin oxidase/dehydrogenases (*CKX1* and *CKX3*) were disease resistant, clearly indicating the importance of cytokinins as a key factor in clubroot disease development (Siemens *et al.* 2006). Further, various fungal biotrophs

(including oomycete) have been proposed to modify plant metabolism via secretion of cytokinins (Ashby 2000; Walters *et al.* 2008).

Thus we aimed our study at compatible interactions between lettuce (*Lactuca sativa* L., cv. Cobham Green) and an oomycete pathogen of downy mildew (*Bremia lactucae* Regel). Modifications in the photosynthetic machinery in leaf discs of lettuce were studied by chlorophyll fluorescence imaging, in the absence and presence of two aromatic cytokinins (BA or mT) and at two levels of irradiance (PAPER II). We have demonstrated that the pathogen caused accelerated decrease in photosynthetic pigment contents (Figure 15) and induced inhibition of PSII and the associated electron transport (F_v/F_m , Φ_{PSII} ; Figures 16, 17). Reduced rate of photosynthesis observed also other authors on various plants infected with powdery mildew (Gordon & Duniway 1982; Wright *et al.* 1995a; Lebeda *et al.* 2008). It seems that mildew infection can inhibit the photosynthetic processes by various mechanisms, including lower supply of light energy due to covering of the leaf surface by mycelium (Yurina *et al.* 1996) and inhibition of CO₂ influx due to stomata closure (Gordon & Duniway 1982). Powdery mildew can also affect photosynthesis indirectly, via pathogen-induced changes in source-

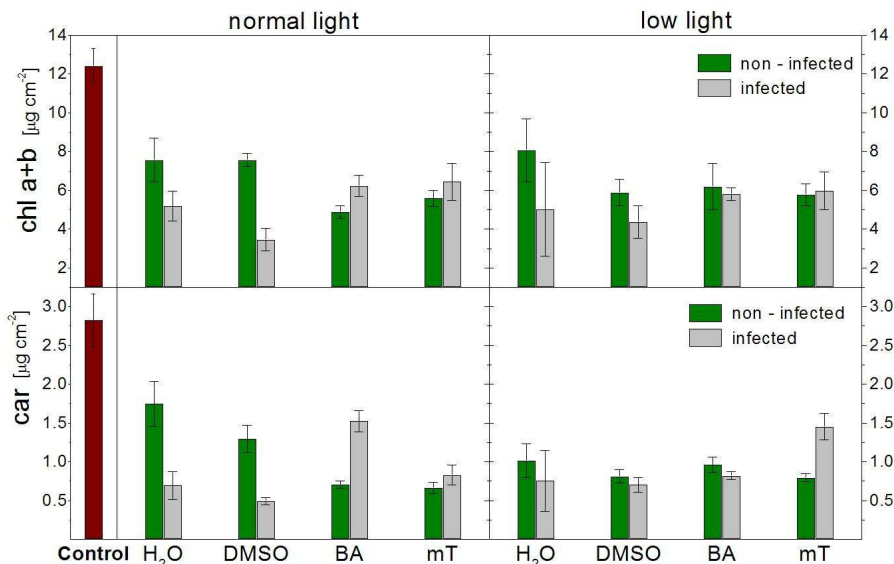


Figure 15. Chlorophyll (a + b) and total carotenoid (car) contents in control lettuce leaves (left), non-infected leaf discs and discs infected with downy mildew, 13 days post inoculation. The discs were incubated under normal and low light (100 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) in water, DMSO, BA or mT solutions. Means and SD are shown, $n = 5$.

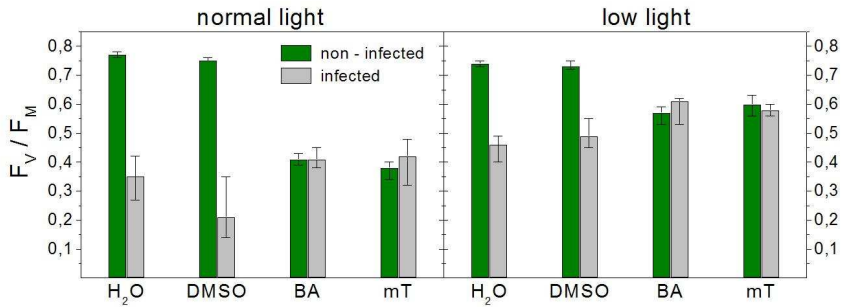


Figure 16. Maximum quantum yield of photosystem II photochemistry (F_v/F_m) in non-infected lettuce leaf discs and discs infected with downy mildew, 13 days post inoculation, incubated under normal and low light (100 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) in water, DMSO, BA or mT solutions. Medians and quartiles are shown, $n = 16$.

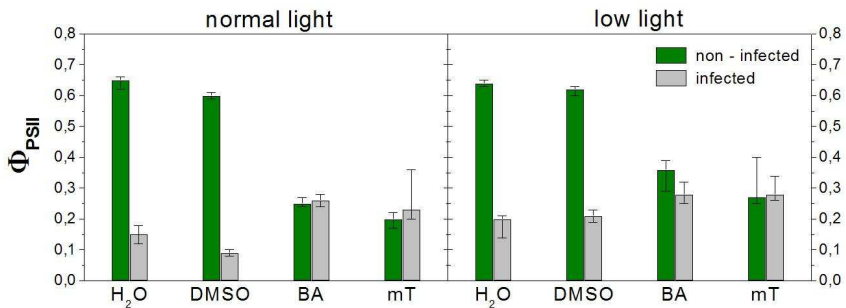


Figure 17. Steady-state values of the actual quantum yield of photosystem II electron transport (Φ_{PSII}) in non-infected lettuce leaf discs and discs infected with downy mildew 13 days post inoculation, incubated under normal and low light (100 and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) in water, DMSO, BA or mT solutions. Medians and quartiles are shown, $n = 8$.

sink relations and nutrient remobilization towards infection sites (Scholes *et al.* 1994; Wright *et al.* 1995b; Abood & Lösel 2003). This can result in inhibition of the Calvin cycle (Gordon & Duniway 1982; Scholes *et al.* 1994; Wright *et al.* 1995a) and in inhibition of photosynthetic light reactions in thylakoid membranes (Magyarosy *et al.* 1976; Moll *et al.* 1995). It has been suggested that the down-regulation of photosynthesis during powdery mildew infection is caused by higher activity of cell-wall invertase, which leads to accumulation of hexose sugars (Scholes *et al.* 1994; Wright *et al.* 1995b; Swarbrick *et al.* 2006) and subsequently to a feed-back inhibition of expression of some photosynthetic genes

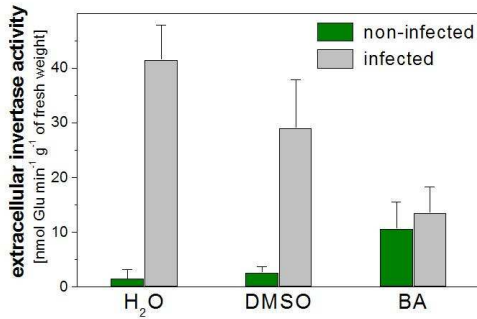


Figure 18. Extracellular invertase activity in non-infected lettuce leaf discs and discs infected with downy mildew 13 days post inoculation, incubated in water, DMSO, BA or mT solutions (at light of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The mean + SD of 3–4 measurements are shown.

(especially *CAB* and *rbcS*) (Scholes *et al.* 1994; Fotopoulos *et al.* 2003; Swarbrick *et al.* 2006). Our results supported the proposed activation of the extracellular invertase also by downy mildew (Figure 18) and further reports showed that the downy mildew induction of invertase activity resulted in the feedback-inhibition of the Calvin cycle and of non-cyclic electron transport as well (Tang *et al.* 1996; Walters & McRoberts 2006).

The exogenous application of both cytokinins used in our experiments had a dual effect. In infected tissues it retarded downy mildew development and restored functionality of photosynthetic processes; in healthy tissues, however, it impaired photosynthetic parameters similar to downy mildew infection. Our experiments have indicated that these contradictory results might have been explained by the cytokinin induction of extracellular invertase activity (Figure 18) and consequent increased hexose accumulation (see Chapter 3.2 Light influences the result of cytokinin action and *vice versa*). Our premise is supported by findings of Ehness, Roitsch, Lara and their other colleagues (Ehness & Roitsch 1997; Lara *et al.* 2004). Thus, cytokinins and the pathogen acting separately had similar effects. Nevertheless, contact of the pathogen with exogenously applied cytokinins suppressed the pathogen growth and consequently the negative effects of infection. External applications of cytokinins may thus suppress the pathogen-driven relocation of carbohydrates and conserve energy in infected plants. Our experiments have shown that the exogenous cytokinin suppressed downy mildew sporulation only if applied prior to inoculation (24 h) and in concentrations as high as 2×10^{-4} M. This prerequisite together with the negative effect of such cytokinin concentrations in non-infected tissues reduces potential utility of cytokinin sprays in field conditions.

4.3 Low temperature stress

Many researches were concentrated on the effect of ABA on cold resistance (Gusta *et al.* 2005; Kim 2007), but less is known of effects of cytokinins as well as of the other plant growth regulators on cold resistance. Nevertheless, the fact that low temperature often induces fast decrease in the shoot cytokinin content reflects possible significance of cytokinin in the cold acclimation. A dramatic decrease in the content of endogenous zeatin-type cytokinins, both in the apical shoot tissues and in the xylem sap after chilling treatment observed e.g. Lejeune *et al.* (1998) in a cold sensitive maize (*Zea mays* L.). Low temperature stress strongly reduced the cytokinin content also in chilling sensitive plants of *Euphorbia pulcherrima*, while in chilling tolerant plants was observed only a slight drop (Tantau & Dorffling 1991). Decrease in the cytokinin content was also observed on wheat plants shortly after the start of root cooling (Veselov *et al.* 2002; Veselova *et al.* 2005; 2006). With presumable mechanism of the cytokinin decrease came Veselova *et al.* (2005; 2006) who suggested that such a fast decrease in cytokinin content was caused mainly by their degradation by cytokinin oxidase/dehydrogenase and further by their lower export rate from chilled roots (Veselova *et al.* 2005; 2006). Furthermore, Li *et al.* (2000) concluded that lower levels of active cytokinins under cold stress may have resulted from its conjugation by zeatin *O*-glycosyltransferases which they observed to accumulate in *Phaseolus vulgaris* and *Zea mays* seedlings under cold stress. Despite the most often observed drop in cytokinin level under cold treatment, there exists evidence of another tendency. One day low temperature treatment did not significantly change the total amount of cytokinins (Z, iP, DZ) in *Arabidopsis* plants (Todorova *et al.* 2005); however, the decrease in total amount of cytokinins was observed after 24 h of recovery under normal growth conditions, and after 2 and 5 day recovery of these plants there were observed marked increases in the cytokinin contents (Todorova *et al.* 2005).

Other publications document consequences of cytokinin treatment in cold tolerance. Exogenous application of cytokinins on beans (Taspinar *et al.* 2009), sugarbeet (Dix *et al.* 1994) or Manila grass (Wang *et al.* 2009), as well as the enhanced levels of endogenous cytokinin in *ipt*-transgenic tall fescue (Hu *et al.* 2005) resulted in enhanced cold tolerance. On the other hand, application of BA on woody plants of saskatoon berry markedly decreased their cold hardiness (Baldwin *et al.* 1998). A negative effect of cytokinin application observed also Veselova and her colleagues. The chilling induced decrease in shoot endogenous cytokinin content may have been responsible for observed closing of stomata in wheat seedlings (Veselova *et al.* 2005; 2006). Pre-treatment with synthetic

cytokinin BA albeit slowed the decline in the transpiration of plants with cooled roots, however, consequently it brought about a visible loss of turgor and wilting (Veselova *et al.* 2005). Turner *et al.* (2001) studied the effects of plant growth regulators on survival, recovery and post-recovery growth of the *Anigozanthos viridis* shoot apices following cryopreservation. They observed that survival of shoot apices was not correlated to cytokinin treatments administered in culture media prior to cryostorage. In recovery media, the application of cytokinins in combination with a gibberellin (GA(3)) was appropriate for obtaining vigorously growing plantlets following cryopreservation. This combination proved to be more effective than basal medium, or *trans*-zeatin and kinetin acting separately (Turner *et al.* 2001).

Further positive effects of cytokinins during the cold treatment were observed on plant growth. Low temperature restrains plant growth of *Arabidopsis thaliana* by a prolonged duration of cell cycle. During the cold treatment, both *amp1* plants with an elevated cytokinin level, and wild-type plants treated with exogenous cytokinins, displayed relative growth rates greater than untreated wild-type plants due to an increase in the total cell number (Xia *et al.* 2009). Chilling treatment given just before the floral transition caused the topmost ear abortion and replacing by a sterile, leaf-like structure. Exogenous application of BA prevented the abortive response caused by chilling (Lejeune *et al.* 1998).

To sum up, cytokinins may have a potential for increasing plant yield under low temperature conditions. Photosynthesis, being a source of plant energy, belongs to the most required processes for plant growth. Although chloroplasts come under the main targets of cytokinin action, studies on the effects of cytokinins on photosynthetic apparatus during cold stress and acclimation are very limited. Accordingly, the next goal of my work was an investigation of chloroplasts modifications during low temperature acclimation, and consequently the influence of cytokinins on it. On the basis of our investigation (PAPER III) we came to a conclusion that the function of photosynthetic pigment protein complexes (PSI and PSII) depends on physical properties as well as on the lipid composition of thylakoid membrane, both of which are affected by low temperature. We demonstrated that cold treatment induced a decrease in the content of a non-bilayer forming lipid monogalactosyl-diacylglycerol (MGDG) in thylakoid membranes of *Arabidopsis thaliana* (Figure 19). The fact that accumulation of this galactolipid requires cytokinins in addition to light (Yamaryo *et al.* 2003; Qi *et al.* 2004) suggests that cytokinins can also influence photosynthetic machinery in this way. Nevertheless, as far as I know there have

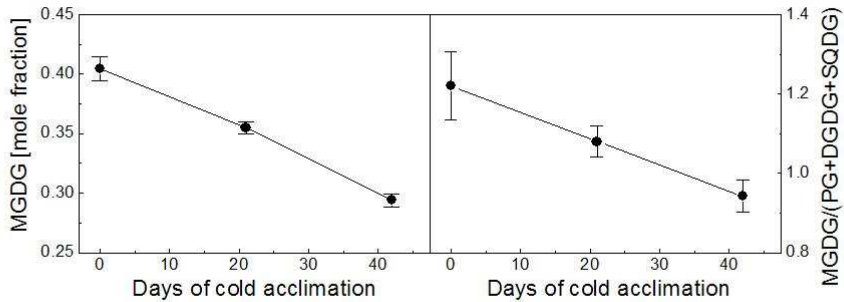


Figure 19. Monogalactosyl-diacylglycerol (MGDG) mole fraction of the total lipid content of chloroplast isolated from *Arabidopsis thaliana* plants subjected to cold acclimation. $MGDG/(PG+DGDG+SQDG)$ represents the ratio of non-bilayer-to-bilayer-forming lipids in chloroplasts (PG, phosphatidylglycerol; DGDG, digalactosyl-diacylglycerol; SQDG, sulfoquinovosyldiacylglycerol). Each point is the mean \pm SE of three or four individual determinations.

not been published any closer relationships between cytokinins and photosynthetic function during cold acclimation yet. Thus investigation pointed at this direction may contribute considerably to our knowledge.

4.4 Conclusion

The molecular mechanism of cytokinin action has not been fully understood yet and gaps in our knowledge project questions in the field of cytokinin influence over plants dealing with stress conditions as well. Although drought stress belongs to the best documented adverse environmental conditions in connection with cytokinins there still remain a lot of questions to be answered. Nevertheless, it seems that cytokinin application may help agriculturalists to reduce losses caused by drought.

Cytokinin applications seem to be promising also for reduction of losses caused by pathogenic invasions. Our results, however, showed that the conditions needed for successful inhibition of pathogen growth (requirement of high cytokinin concentration together with the necessity of cytokinin application preceding the inoculation) highly reduce the possible utility of cytokinins as disease control agents, at least for lettuce (*Lactuca sativa* L.) endangered by the biotrophic oomycete pathogen *Bremia lactucae* Regel (PAPER II).

There exists evidence of the positive effect of cytokinin application on plant cold hardiness but studies concerning this topic are limited. Thus, investigation focused on cytokinin influence on plants suffering cold stress may bring new results with implications for agronomists. From a few available reports

on cytokinin effects on chloroplasts during cold stress it is obvious that cytokinins can influence lipid composition of thylakoid membranes. Thus, my further research aimed initially at changes in thylakoid membrane properties during low temperature treatment (PAPER III). I have also performed some pre-experiments pointed at the influence of endogenously elevated cytokinin level on cold acclimation; however, there is still a long way before I can contrive the results into a publishable shape. And here I also see one of exciting challenges for my future work.

5 References

- Abood, J.K. & Lösel, D.M. (2003) Changes in carbohydrate composition of cucumber leaves during the development of powdery mildew infection. *Plant Pathology* **52**, 256-265.
- Ahmad, M. & Cashmore, A.R. (1997) The blue-light receptor cryptochrome 1 shows functional dependence on phytochrome A or phytochrome B in *Arabidopsis thaliana*. *Plant Journal* **11**, 421-427.
- Ananieva, K., Ananiev, E.D., Doncheva, S., Georgieva, K., Tzvetkova, N., Kamínek, M., Motyka, V., Dobrev, P., Gajdošová, S. & Malbeck, J. (2008) Senescence progression in a single darkened cotyledon depends on the light status of the other cotyledon in *Cucurbita pepo* (zucchini) seedlings: potential involvement of cytokinins and cytokinin oxidase/dehydrogenase activity. *Physiologia Plantarum* **134**, 609-623.
- Ananieva, K., Malbeck, J., Kamínek, M. & van Staden, J. (2004) Changes in endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) during natural and dark-induced senescence. *Physiologia Plantarum* **122**, 133-142.
- Ang, L.H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A. & Deng, X.W. (1998) Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Molecular Cell* **1**, 213-222.
- Argueso, C.T., Ferreira, F.J. & Kieber, J.J. (2009) Environmental perception avenues: the interaction of cytokinin and environmental response pathways. *Plant Cell and Environment* **32**, 1147-1160.
- Ashby, A.M. (2000) Biotrophy and the cytokinin conundrum. *Physiological and Molecular Plant Pathology* **57**, 147-158.
- Baldwin, B.D., Bandara, M.S. & Tanino, K.K. (1998) Is tissue culture a viable system with which to examine environmental and hormonal regulation of cold acclimation in woody plants? *Physiologia Plantarum* **102**, 201-209.
- Bari, R. & Jones, J. (2009) Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69**, 473-488.
- Benková, E., Witters, E., Van Dongen, W., Kolář, J., Motyka, V., Brzobohatý, B., Van Onckelen, H.A. & Macháček, I. (1999) Cytokinins in tobacco and wheat chloroplasts, occurrence and changes due to light/dark treatment. *Plant Physiology* **121**, 245-251.
- Boonman, A., Prinsen, E., Gilmer, F., Schurr, U., Peeters, A.J.M., Voeselek, L. & Pons, T.L. (2007) Cytokinin import rate as a signal for photosynthetic acclimation to canopy light gradients. *Plant Physiology* **143**, 1841-1852.
- Boonman, A., Prinsen, E., Voeselek, L. & Pons, T.L. (2009) Redundant roles of photoreceptors and cytokinins in regulating photosynthetic acclimation to canopy density. *Journal of Experimental Botany* **60**, 1179-1190.
- Bracale, M., Longo, G.P., Rossi, G. & Longo, C.P. (1988) Early changes in morphology and polypeptide pattern of plastids from watermelon cotyledons induced by benzyladenine or light are very similar. *Physiologia Plantarum* **72**, 94-100.

- Brault, M. & Maldiney, R. (1999) Mechanisms of cytokinin action. *Plant Physiology and Biochemistry* **37**, 403-412.
- Buschmann, C. & Lichtenthaler, H.K. (1982) The effect of cytokinins on growth and pigment accumulation of radish seedlings (*Raphanus sativus* L.) grown in the dark and at different light quanta fluence rates. *Photochemistry and Photobiology* **35**, 217-221.
- Cag, S., Palavan-Ünsal, N. & Büyüktuncer, D. (2003) Comparison of the effects of *meta*-topolin and other cytokinins on chlorophyll and protein contents and peroxidase activity in cucumber cotyledons. *Israel Journal of Plant Sciences* **51**, 261-265.
- Carimi, F., Terzi, M., De Michele, R., Zottini, M. & Lo Schiavo, F. (2004) High levels of the cytokinin BAP induce PCD by accelerating senescence. *Plant Science* **166**, 963-969.
- Carimi, F., Zottini, M., Formentin, E., Terzi, M. & Lo Schiavo, F. (2003) Cytokinins: new apoptotic inducers in plants. *Planta* **216**, 413-421.
- Čatský, J., Pospíšilová, J., Kamínek, M., Gaudinová, A., Pulkrábek, J. & Zahradníček, J. (1996) Seasonal changes in sugar beet photosynthesis as affected by exogenous cytokinin N-6-(m-hydroxybenzyl)adenosine. *Biologia Plantarum* **38**, 511-518.
- Chen, D.Q., Li, Z.Y., Pan, R.C. & Wang, X.J. (2006) Anthocyanin accumulation mediated by blue light and cytokinin in Arabidopsis seedlings. *Journal of Integrative Plant Biology* **48**, 420-425.
- Chernyad'ev, II (1995) Photosynthesis in water-stressed wheat – protective effects of cytokinins. *Applied Biochemistry and Microbiology* **31**, 554-559.
- Chernyad'ev, II (2000) Ontogenetic changes in the photosynthetic apparatus and effects of cytokinins (review). *Applied Biochemistry and Microbiology* **36**, 527-539.
- Chernyad'ev, II (2002) Effect of preparations exhibiting cytokinin-like activity on the specific density of leaf in grasses. *Applied Biochemistry and Microbiology* **38**, 593-601.
- Chernyad'ev, II (2005) Effect of water stress on the photosynthetic apparatus of plants and the protective role of cytokinins: A review. *Applied Biochemistry and Microbiology* **41**, 115-128.
- Chernyad'ev, II (2009) The protective action of cytokinins on the photosynthetic machinery and productivity of plants under stress (review). *Applied Biochemistry and Microbiology* **45**, 351-362.
- Chernyad'ev, II & Monakhova, O.F. (1998) The activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase in wheat plants as affected by water stress and kartolin-4. *Photosynthetica* **35**, 603-610.
- Chernyad'ev, II & Monakhova, O.F. (2001) Activity of carbon metabolism enzymes in wheat plants treated with kartolin-4 and exposed to water stress. *Applied Biochemistry and Microbiology* **37**, 603-609.
- Chernyad'ev, II & Monakhova, O.F. (2003) Effects of cytokinin preparations on the pools of pigments and proteins of wheat cultivars differing in their tolerance to water stress. *Applied Biochemistry and Microbiology* **39**, 524-531.

- Chernyad'ev, II & Monakhova, O.F. (2006) Activity of NADP-dependent glyceraldehyde-phosphate dehydrogenase and phosphoenolpyruvate carboxylase in wheat leaves under water stress. *Applied Biochemistry and Microbiology* **42**, 312-319.
- Chin-Atkins, A.N., Craig, S., Hocart, C.H., Dennis, E.S. & Chaudhury, A.M. (1996) Increased endogenous cytokinin in the *Arabidopsis amp1* mutant corresponds with de-etiolation responses. *Planta* **198**, 549-556.
- Choi, J. & Hwang, I. (2007) Cytokinin: Perception, signal transduction, and role in plant growth and development. *Journal of Plant Biology* **50**, 98-108.
- Chory, J. (2010) Light signal transduction: an infinite spectrum of possibilities. *Plant Journal* **61**, 982-991.
- Chory, J., Reinecke, D., Sim, S., Washburn, T. & Brenner, M. (1994) A role for cytokinins in de-etiolation in *Arabidopsis - det* mutants have an altered response to cytokinins. *Plant Physiology* **104**, 339-347.
- Choudhury, N.K. & Choe, H.T. (1996) Photoprotective effect of kinetin on pigment content and photochemical activities of wheat chloroplasts aging in vitro. *Biologia Plantarum* **38**, 61-69.
- Clark, D.G., Dervinis, C., Barret, J.E., Klee, H. & Jones, M. (2004) Drought-induced leaf senescence and horticultural performance of transgenic P_{SAG12}-IPT petunias. *Journal of the American Society for Horticultural Science* **129**, 93-99.
- Cluis, C.P., Mouchel, C.F. & Hardtke, C.S. (2004) The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. *Plant Journal* **38**, 332-347.
- Dertinger, U., Schaz, U. & Schulze, E.D. (2003) Age-dependence of the antioxidative system in tobacco with enhanced glutathione reductase activity or senescence-induced production of cytokinins. *Physiologia Plantarum* **119**, 19-29.
- Dhindsa, R.S., Plumbdhindsa, P.L. & Reid, D.M. (1982) Leaf senescence and lipid-peroxidation – effects of some phytohormones, and scavengers of free-radicals and singlet oxygen. *Physiologia Plantarum* **56**, 453-457.
- Dix, P.J., Finch, I. & Burke, J.I. (1994) Genotypic differences in cold tolerance are masked by high sucrose and cytokinin in shoot cultures of sugarbeet. *Plant Cell Tissue and Organ Culture* **36**, 285-290.
- Durmuş, N. & Kadioğlu, A. (1998) Effect of benzyladenine on peroxidase activity during senescence of sunflower (*Helianthus annuus* L.) cotyledons. *Phyton-Annales Rei Botanicae* **37**, 253-261.
- Ehness, R. & Roitsch, T. (1997) Co-ordinated induction of mRNAs for extracellular invertase and a glucose transporter in *Chenopodium rubrum* by cytokinins. *Plant Journal* **11**, 539-548.
- Ferreira, F.J. & Kieber, J.J. (2005) Cytokinin signaling. *Current Opinion in Plant Biology* **8**, 518-525.
- Fotopoulos, V., Gilbert, M.J., Pittman, J.K., Marvier, A.C., Buchanan, A.J., Sauer, N., Hall, J.L. & Williams, L.E. (2003) The monosaccharide transporter gene, *AtSTP4*, and the cell-wall invertase, *Atβfruct1*, are induced

- in *Arabidopsis* during infection with the fungal biotroph *Erysiphe cichoracearum*. *Plant Physiology* **132**, 821-829.
- Franco-Zorrilla, J.M., Martín, A.C., Leyva, A. & Par-Ares, J.P. (2005) Interaction between phosphate-starvation, sugar, and cytokinin signaling in *Arabidopsis* and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiology* **138**, 847-857.
- Franklin, K.A. & Quail, P.H. (2010) Phytochrome functions in *Arabidopsis* development. *Journal of Experimental Botany* **61**, 11-24.
- Gan, S.S. & Amasino, R.M. (1995) Inhibition of leaf senescence by autoregulated production of cytokinin. *Science* **270**, 1986-1988.
- Gan, S.S. & Amasino, R.M. (1996) Cytokinins in plant senescence: From spray and pray to clone and play. *Bioessays* **18**, 557-565.
- Gan, S.S. & Amasino, R.M. (1997) Making sense of senescence – Molecular genetic regulation and manipulation of leaf senescence. *Plant Physiology* **113**, 313-319.
- Gao, Y., Li, J.M., Strickland, E., Hua, S.J., Zhao, H.Y., Chen, Z.L., Qu, L.J. & Deng, X.W. (2004) An *Arabidopsis* promoter microarray and its initial usage in the identification of HY5 binding targets *in vitro*. *Plant Molecular Biology* **54**, 683-699.
- Genkov, T., Tsoneva, P. & Ivanova, I. (1997) Effect of cytokinins on photosynthetic pigments and chlorophyllase activity in *in vitro* cultures of axillary buds of *Dianthus caryophyllus* L. *Journal of Plant Growth Regulation* **16**, 169-172.
- Gordon, T.R. & Duniway, J.M. (1982) Effects of powdery mildew infection on the efficiency of CO₂ fixation and light utilization by sugar beet leaves. *Plant Physiology* **69**, 139-142.
- Gusta, L.V., Trischuk, R. & Weiser, C.J. (2005) Plant cold acclimation: The role of abscisic acid. *Journal of Plant Growth Regulation* **24**, 308-318.
- Haisel, D., Pospíšilová, J., Synková, H., Schnablová, R. & Baťková, P. (2006) Effects of abscisic acid or benzyladenine on pigment contents, chlorophyll fluorescence, and chloroplast ultrastructure during water stress and after rehydration. *Photosynthetica* **44**, 606-614.
- Halliday, K.J. & Fankhauser, C. (2003) Phytochrome-hormonal signalling networks. *New Phytologist* **157**, 449-463.
- Hammerton, R.D., Nicander, B. & Tillberg, E. (1998) Irradiance-induced alterations of growth and cytokinins in *Phaseolus vulgaris* seedlings. *Plant Growth Regulation* **25**, 63-69.
- Hare, P.D., Cress, W.A. & van Staden, J. (1997) The involvement of cytokinins in plant responses to environmental stress. *Plant Growth Regulation* **23**, 79-103.
- He, P., Osaki, M., Takebe, M., Shinano, T. & Wasaki, J. (2005) Endogenous hormones and expression of senescence-related genes in different senescent types of maize. *Journal of Experimental Botany* **56**, 1117-1128.
- Hidema, J., Makino, A., Kurita, Y., Mae, T. & Ojima, K. (1992) Changes in the levels of chlorophyll and light-harvesting chlorophyll *a/b* protein of PSII in

- rice leaves aged under different irradiances from full expansion through senescence. *Plant and Cell Physiology* **33**, 1209-1214.
- Higuchi, M., Pischke, M.S., Mähönen, A.P., Miyawaki, K., Hashimoto, Y., Seki, M., Kobayashi, M., Shinozaki, K., Kato, T., Tabata, S., Helariutta, Y., Sussman, M.R. & Kakimoto, T. (2004) *In planta* functions of the *Arabidopsis* cytokinin receptor family. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 8821-8826.
- Hirose, N., Takei, K., Kuroha, T., Kamada-Nobusada, T., Hayashi, H. & Sakakibara, H. (2008) Regulation of cytokinin biosynthesis, compartmentalization and translocation. *Journal of Experimental Botany* **59**, 75-83.
- Hoecker, U. & Quail, P.H. (2001) The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in *Arabidopsis*. *Journal of Biological Chemistry* **276**, 38173-38178.
- Holub, J., Hanuš, J., Hanke, D.E. & Strnad, M. (1998) Biological activity of cytokinins derived from *ortho*- and *meta*-hydroxybenzyladenine. *Plant Growth Regulation* **26**, 109-115.
- Hu, Y.L., Jia, W.L., Wang, J.D., Zhang, Y.Q., Yang, L.L. & Lin, Z.P. (2005) Transgenic tall fescue containing the *Agrobacterium tumefaciens ipt* gene shows enhanced cold tolerance. *Plant Cell Reports* **23**, 705-709.
- Huang, F.Y., PhilosophHadas, S., Meir, S., Callahan, D.A., Sabato, R., Zelcer, A. & Hepler, P.K. (1997) Increases in cytosolic Ca²⁺ in parsley mesophyll cells correlate with leaf senescence. *Plant Physiology* **115**, 51-60.
- Hudák, J., Vizárová, G., Šikulová, J. & Ovečková, O. (1996) Effect of cytokinins produced by strains of *Agrobacterium tumefaciens* with binary vectors on plastids in senescent barley leaves. *Acta Physiologiae Plantarum* **18**, 205-210.
- Hung, K.T. & Kao, C.H. (1998) Involvement of lipid peroxidation in methyl jasmonate-promoted senescence in detached rice leaves. *Plant Growth Regulation* **24**, 17-21.
- Hwang, D., Chen, H.C. & Sheen, J. (2002) Two-component signal transduction pathways in *Arabidopsis*. *Plant Physiology* **129**, 500-515.
- Hwang, I. & Sakakibara, H. (2006) Cytokinin biosynthesis and perception. *Physiologia Plantarum* **126**, 528-538.
- Hwang, I. & Sheen, J. (2001) Two-component circuitry in *Arabidopsis* cytokinin signal transduction. *Nature* **413**, 383-389.
- Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., Tabata, S., Shinozaki, K. & Kakimoto, T. (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* **409**, 1060-1063.
- Ivanova, A.P., Stefanov, K.L. & Yordanov, I.T. (1998) Effect of cytokinin 4-PU-30 on the lipid composition of water stressed bean plants. *Biologia Plantarum* **41**, 155-159.
- Kakimoto, T. (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* **274**, 982-985.

- Kamada-Nobusada, T. & Sakakibara, H. (2009) Molecular basis for cytokinin biosynthesis. *Phytochemistry* **70**, 444-449.
- Kamínek, M., Vaněk, T. & Motyka, V. (1987) Cytokinin activities of *N*⁶-benzyladenosine derivatives hydroxylated on the side-chain phenyl ring. *Journal of Plant Growth Regulation* **6**, 113-120.
- Kar, M., Streb, P., Hertwig, B. & Feierabend, J. (1993) Sensitivity to photodamage increases during senescence in excised leaves. *Journal of Plant Physiology* **141**, 538-544.
- Kim, J. (2007) Perception, transduction, and networks in cold signaling. *Journal of Plant Biology* **50**, 139-147.
- Klerk, H., Rebers, M. & Vanloon, L.C. (1993) Effects of light and regulators on senescence-related changes in soluble-proteins in detached oat (*Avena sativa* L.) leaves. *Plant Growth Regulation* **13**, 137-145.
- Kraepiel, Y. & Miginiac, E. (1997) Photomorphogenesis and phytohormones. *Plant Cell and Environment* **20**, 807-812.
- Kraus, T.E., Hofstra, G. & Fletcher, R.A. (1993) Regulation of senescence by benzylaminopurine and uniconazole in intact and excised soybean cotyledons. *Plant Physiology and Biochemistry* **31**, 827-834.
- Kreslavski, V.D., Carpentier, R., Klimov, V.V. & Allakhverdiev, S.I. (2009) Transduction mechanisms of photoreceptor signals in plant cells. *Journal of Photochemistry and Photobiology C-Photochemistry Reviews* **10**, 63-80.
- Kumar, B., Pandey, D.M., Goswami, C.L. & Jain, S. (2001) Effect of growth regulators on photosynthesis, transpiration and related parameters in water stressed cotton. *Biologia Plantarum* **44**, 475-478.
- Kurepin, L.V., Emery, R.J.N., Chinnappa, C.C. & Reid, D.M. (2008) Light irradiance differentially regulates endogenous levels of cytokinins and auxin in alpine and prairie genotypes of *Stellaria longipes*. *Physiologia Plantarum* **134**, 624-635.
- Kusnetsov, V., Landsberger, M., Meurer, J. & Oelmüller, R. (1999) The assembly of the CAAT-box binding complex at a photosynthesis gene promoter is regulated by light, cytokinin, and the stage of the plastids. *Journal of Biological Chemistry* **274**, 36009-36014.
- Kusnetsov, V.V., Oelmüller, R., Sarwat, M.I., Porfirova, S.A., Cherepneva, G.N., Herrmann, R.G. & Kulaeva, O.N. (1994) Cytokinins, abscisic-acid and light affect accumulation of chloroplast proteins in *Lupinus luteus* cotyledons without notable effect on steady-state mRNA levels – Specific protein response to light/phytohormone interaction. *Planta* **194**, 318-327.
- Lara, M.E.B., Garcia, M.C.G., Fatima, T., Ehness, R., Lee, T.K., Proels, R., Tanner, W. & Roitsch, T. (2004) Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. *Plant Cell* **16**, 1276-1287.
- Lebeda, A., Sedlářová, M., Petřivalský, M. & Prokopová, J. (2008) Diversity of defence mechanisms in plant-oomycete interactions: a case study of *Lactuca* spp. and *Bremia lactucae*. *European Journal of Plant Pathology* **122**, 71-89.

- Lejeune, P., Prinsen, E., Van Onckelen, H. & Bernier, G. (1998) Hormonal control of ear abortion in a stress-sensitive maize (*Zea mays*) inbred. *Australian Journal of Plant Physiology* **25**, 481-488.
- Lerbs, S., Lerbs, W., Klyachko, N.L., Romanko, E.G., Kulaeva, O.N., Wollgiehn, R. & Parthier, B. (1984) Gene-expression in cytokinin- and light-mediated plastogenesis of *Cucurbita* cotyledons – ribulose-1,5-bisphosphate carboxylase/oxygenase. *Planta* **162**, 289-298.
- Letham, D.S. (1963) Zeatin, a factor inducing cell division isolated from *Zea mays*. *Life Sciences*, 569-573.
- Li, R., Sosa, J.L. & Zavala, M.E. (2000) Accumulation of zeatin O-glycosyltransferase in *Phaseolus vulgaris* and *Zea mays* following cold stress. *Plant Growth Regulation* **32**, 295-305.
- Liu, X.H. & Huang, B.R. (2002) Cytokinin effects on creeping bentgrass response to heat stress: 2. Leaf senescence and antioxidant metabolism. *Crop Science* **42**, 466-472.
- Ma, Q.H. (2008) Genetic engineering of cytokinins and their application to agriculture. *Critical Reviews in Biotechnology* **28**, 213-232.
- Macháčková, I., Krekule, J., Eder, J., Seidlová, F. & Strnad, M. (1993) Cytokinins in photoperiodic induction of flowering in *Chenopodium* species. *Physiologia Plantarum* **87**, 160-166.
- Magyarosy, A.C., Schürmann, P. & Buchanan, B.B. (1976) Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. *Plant Physiology* **57**, 486-489.
- Metwally, A., Tsonev, T. & Zeinalov, Y. (1997) Effect of cytokinins on the photosynthetic apparatus in water-stressed and rehydrated bean plants. *Photosynthetica* **34**, 563-567.
- Michniewicz, M., Rozej, B. & Kruszka, G. (1984) Control of growth and development of isolates of *Fusarium culmorum* (W.G.Sm) Sacc. of different pathogenicity to wheat seedlings by plant growth regulators. 3. cytokinins. *Acta Physiologiae Plantarum* **6**, 3-11.
- Miller, C.O., Skoog, F., von Saltza, M.H. & Strong, F.M. (1955) Kinetin, a cell division factor from deoxyribonucleic acid. *Journal of the American Chemical Society* **77**, 1392-1392.
- Mills, P.R., Gussin, E.J. & Wood, R.K.S. (1986) Induction of resistance in cucumber to *Colletotrichum lagenarium* by 6-benzylaminopurine. *Journal of Phytopathology–Phytopathologische Zeitschrift* **116**, 11-17.
- Mira-Rodado, V., Sweere, U., Grefen, C., Kunkel, T., Fejes, E., Nagy, F., Schäfer, E. & Harter, K. (2007) Functional cross-talk between two-component and phytochrome B signal transduction in *Arabidopsis*. *Journal of Experimental Botany* **58**, 2595-2607.
- Mishina, G.N., Talieva, M.N., Babosha, A.V., Serezhkina, G.V. & Andreev, L.N. (2002) Influence of phytohormones on development of conidial inoculum of causative agents of the phlox and barley powdery mildew. *Biology Bulletin* **29**, 46-52.

- Mlejnek, P., Doležel, P. & Procházka, S. (2003) Intracellular phosphorylation of benzyladenosine is related to apoptosis induction in tobacco BY-2 cells. *Plant Cell and Environment* **26**, 1723-1735.
- Mlejnek, P. & Procházka, S. (2002) Activation of caspase-like proteases and induction of apoptosis by isopentenyladenosine in tobacco BY-2 cells. *Planta* **215**, 158-166.
- Mok, D.W.S. & Mok, M.C. (2001) Cytokinin metabolism and action. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 89-118.
- Moll, S., Serrano, P. & Boyle, C. (1995) *In vivo* chlorophyll fluorescence in rust-infected bean plants. *Journal of Applied Botany-Angewandte Botanik* **69**, 163-168.
- Munné-Bosch, S. & Alegre, L. (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* **31**, 203-216.
- Mähönen, A.P., Bishopp, A., Higuchi, M., Nieminen, K.M., Kinoshita, K., Törmäkangas, K., Ikeda, Y., Oka, A., Kakimoto, T. & Helariutta, Y. (2006a) Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* **311**, 94-98.
- Mähönen, A.P., Higuchi, M., Thörmäkangas, K., Miyawaki, K., Pischke, M.S., Sussman, M.R., Helariutta, Y. & Kakimoto, T. (2006b) Cytokinins regulate a bidirectional phosphorelay network in *Arabidopsis*. *Current Biology* **16**, 1116-1122.
- Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S. & Ueguchi, C. (2004) Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in *Arabidopsis*. *Plant Cell* **16**, 1365-1377.
- Nogué, N., Hocart, H., Letham, D.S., Dennis, E.S. & Chaudhury, A.M. (2000) Cytokinin synthesis is higher in the *Arabidopsis ampl* mutant. *Plant Growth Regulation* **32**, 267-273.
- Noodén, L.D., Guiamet, J.J. & John, I. (1997) Senescence mechanisms. *Physiologia Plantarum* **101**, 746-753.
- Nováková, M., Motyka, V., Dobrev, P.I., Malbeck, J., Gaudinová, A. & Vanková, R. (2005) Diurnal variation of cytokinin, auxin and abscisic acid levels in tobacco leaves. *Journal of Experimental Botany* **56**, 2877-2883.
- Nozue, K. & Maloof, J.N. (2006) Diurnal regulation of plant growth. *Plant Cell and Environment* **29**, 396-408.
- Osakabe, Y., Miyata, S., Urao, T., Seki, M., Shinozaki, K. & Yamaguchi-Shinozaki, K. (2002) Overexpression of *Arabidopsis* response regulators, ARR4/ATRR1/IBC7 and ARR8/ATRR3, alters cytokinin responses differentially in the shoot and in callus formation. *Biochemical and Biophysical Research Communications* **293**, 806-815.
- Paramonova, N.V., Krasavina, M.S. & Sokolova, S.V. (2002) Ultrastructure of chloroplasts in phloem companion cells and mesophyll cells as related to the stimulation of sink activity by cytokinins. *Russian Journal of Plant Physiology* **49**, 187-195.

- Perilli, S., Moubayidin, L. & Sabatini, S. (2010) The molecular basis of cytokinin function. *Current Opinion in Plant Biology* **13**, 21-26.
- Petit-Paly, G., Franck, T., Brisson, L., Kevers, C., Chenieux, J.C. & Rideau, M. (1999) Cytokinin modulates catalase activity and coumarin accumulation in *in vitro* cultures of tobacco. *Journal of Plant Physiology* **155**, 9-15.
- Pons, T.L. & Bergkotte, M. (1996) Nitrogen allocation in response to partial shading of a plant: Possible mechanisms. *Physiologia Plantarum* **98**, 571-577.
- Pons, T.L., Jordi, W. & Kuiper, D. (2001) Acclimation of plants to light gradients in leaf canopies: evidence for a possible role for cytokinins transported in the transpiration stream. *Journal of Experimental Botany* **52**, 1563-1574.
- Pospíšilová, J. (2003a) Interaction of cytokinins and abscisic acid during regulation of stomatal opening in bean leaves. *Photosynthetica* **41**, 49-56.
- Pospíšilová, J. (2003b) Participation of phytohormones in the stomatal regulation of gas exchange during water stress. *Biologia Plantarum* **46**, 491-506.
- Pospíšilová, J. & Bařková, P. (2004) Effects of pre-treatments with abscisic acid and/or benzyladenine on gas exchange of French bean, sugar beet, and maize leaves during water stress and after rehydration. *Biologia Plantarum* **48**, 395-399.
- Pospíšilová, J., Rulcová, J. & Vomáčka, L. (2001) Effect of benzyladenine and hydroxybenzyladenosine on gas exchange of bean and sugar beet leaves. *Biologia Plantarum* **44**, 523-528.
- Pospíšilová, J., Synková, H., Macháčková, I. & Čatský, J. (1998) Photosynthesis in different types of transgenic tobacco plants with elevated cytokinin content. *Biologia Plantarum* **40**, 81-89.
- Pospíšilová, J., Synková, H. & Rulcová, J. (2000) Cytokinins and water stress. *Biologia Plantarum* **43**, 321-328.
- Pospíšilová, J., Vágner, M., Malbeck, J., Trávníčková, A. & Bařková, P. (2005) Interactions between abscisic acid and cytokinins during water stress and subsequent rehydration. *Biologia Plantarum* **49**, 533-540.
- Pospíšilová, J., Čatský, J., Synková, H., Macháčková, I. & Solárová, J. (1993) 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.
- Procházková, D. & Wilhelmová, N. (2004) Changes in antioxidative protection in bean cotyledons during natural and continuous irradiation-accelerated senescence. *Biologia Plantarum* **48**, 33-39.
- Qi, Y.H., Yamauchi, Y., Ling, J.Q., Kawano, N., Li, D.B. & Tanaka, K. (2004) Cloning of a putative monogalactosyldiacylglycerol synthase gene from rice (*Oryza sativa* L.) plants and its expression in response to submergence and other stresses. *Planta* **219**, 450-458.
- Rashotte, A.M., Carson, S.D.B., To, J.P.C. & Kieber, J.J. (2003) Expression profiling of cytokinin action in Arabidopsis. *Plant Physiology* **132**, 1998-2011.
- Rashotte, A.M., Chae, H.S., Maxwell, B.B. & Kieber, J.J. (2005) The interaction of cytokinin with other signals. *Physiologia Plantarum* **123**, 184-194.

- Riefler, M., Novák, O., Strnad, M. & Schmülling, T. (2006) *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell* **18**, 40-54.
- Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. & Blumwald, E. (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19631-19636.
- Rivero, R.M., Shulaev, V. & Blumwald, E. (2009) Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. *Plant Physiology* **150**, 1530-1540.
- Romanov, G.A. (2009) How do cytokinins affect the cell? *Russian Journal of Plant Physiology* **56**, 268-290.
- Rulcová, J. & Pospíšilová, J. (2001) Effect of benzylaminopurine on rehydration of bean plants after water stress. *Biologia Plantarum* **44**, 75-81.
- Saijo, Y., Sullivan, J.A., Wang, H.Y., Yang, J.P., Shen, Y.P., Rubio, V., Ma, L.G., Hoecker, U. & Deng, X.W. (2003) The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes & Development* **17**, 2642-2647.
- Sakai, H., Aoyama, T. & Oka, A. (2000) *Arabidopsis* ARR1 and ARR2 response regulators operate as transcriptional activators. *Plant Journal* **24**, 703-711.
- Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S. & Oka, A. (2001) ARR1, a transcription factor for genes immediately responsive to cytokinins. *Science* **294**, 1519-1521.
- Sakakibara, H. (2006) Cytokinins: Activity, biosynthesis, and translocation. *Annual Review of Plant Biology* **57**, 431-449.
- Sakakibara, H., Takei, K. & Hirose, N. (2006) Interactions between nitrogen and cytokinin in the regulation of metabolism and development. *Trends in Plant Science* **11**, 440-448.
- Salomé, P.A., To, J.P.C., Kieber, J.J. & McClung, C.R. (2006) *Arabidopsis* response regulators ARR3 and ARR4 play cytokinin-independent roles in the control of circadian period. *Plant Cell* **18**, 55-69.
- Sarhan, A.R.T., Király, Z., Sziráki, I. & Smedegaard-Petersen, V. (1991) Increased levels of cytokinins in barley leaves having the systemic acquired-resistance to *Bipolaris-sorokiniana* (Sacc.) Shoemaker. *Journal of Phytopathology-Phytopathologische Zeitschrift* **131**, 101-108.
- Scheres, B., Dilaurenzio, L., Willemsen, V., Hauser, M.T., Janmaat, K., Weisbeek, P. & Benfey, P.N. (1995) Mutations affecting the radial organization of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* **121**, 53-62.
- Schmülling, T. (2002) New insights into the functions of cytokinins in plant development. *Journal of Plant Growth Regulation* **21**, 40-49.
- Schmülling, T., Schäfer, S. & Romanov, G. (1997) Cytokinins as regulators of gene expression. *Physiologia Plantarum* **100**, 505-519.
- Scholes, J.D., Lee, P.J., Horton, P. & Lewis, D.H. (1994) Invertase: understanding changes in the photosynthetic and carbohydrate metabolism

- of barley leaves infected with powdery mildew. *New Phytologist* **126**, 213-222.
- Selivankina, S.Y., Karavaiko, N.N., Kuiper, D., Novikova, G.V. & Kulaeva, O.N. (2001) Cytokinin activity of zeatin allylic phosphate, a natural compound. *Plant Growth Regulation* **33**, 157-164.
- Shao, R.X., Wang, K.B. & Shangguan, Z.P. (2010) Cytokinin-induced photosynthetic adaptability of *Zea mays* L. to drought stress associated with nitric oxide signal: Probed by ESR spectroscopy and fast OJIP fluorescence rise. *Journal of Plant Physiology* **167**, 472-479.
- She, X.P. & Song, X.G. (2006) Cytokinin- and auxin-induced stomatal opening is related to the change of nitric oxide levels in guard cells in broad bean. *Physiologia Plantarum* **128**, 569-579.
- Sheen, J. (2002) Phosphorelay and transcription control in cytokinin signal transduction. *Science* **296**, 1650-1652.
- Siemens, J., Keller, I., Sarx, J., Kunz, S., Schuller, A., Nagel, W., Schümlling, T., Parniske, M. & Ludwig-Müller, J. (2006) Transcriptome analysis of *Arabidopsis* clubroots indicate a key role for cytokinins in disease development. *Molecular Plant-Microbe Interactions* **19**, 480-494.
- Smith, H. (2000) Phytochromes and light signal perception by plants – an emerging synthesis. *Nature* **407**, 585-591.
- Stoyanova, D. & Yordanov, I. (1999) Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of plants. 2. Chloroplast ultrastructure of primary bean leaves. *Photosynthetica* **37**, 621-625.
- Strnad, M., Hanuš, J., Vaněk, T., Kamínek, M., Ballantine, J.A., Fussell, B. & Hanke, D.E. (1997) *Meta-topolin*, a highly active aromatic cytokinin from poplar leaves (*Robusta*). *Phytochemistry* **45**, 213-218.
- Strnad, M., Peters, W., Hanuš, J. & Beck, E. (1994) *Ortho-topolin-9-glucoside*, an aromatic cytokinin from *Populus x canadensis* cv. *Robusta* leaves. *Phytochemistry* **37**, 1059-1062.
- Suzuki, T., Miwa, K., Ishikawa, K., Yamada, H., Aiba, H. & Mizuno, T. (2001) The *Arabidopsis* sensor His-kinase, AHK4, can respond to cytokinins. *Plant and Cell Physiology* **42**, 107-113.
- Swarbrick, P.J., Schulze-Lefert, P. & Scholes, J.D. (2006) Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. *Plant Cell and Environment* **29**, 1061-1076.
- Sweere, U., Eichenberg, K., Lohrmann, J., Mira-Rodado, V., Bäurle, I., Kudla, J., Nagy, F., Schäfer, E. & Harter, K. (2001) Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. *Science* **294**, 1108-1111.
- Synková, H., VanLoven, K. & Valcke, R. (1997a) Increased content of endogenous cytokinins does not delay degradation of photosynthetic apparatus in tobacco. *Photosynthetica* **33**, 595-608.
- Synková, H., Wilhelmová, N., Šesták, Z. & Pospíšilová, J. (1997b) Photosynthesis in transgenic plants with elevated cytokinin content. In:

- Handbook of photosynthesis* (ed. M. Pessaraki), pp. 541-552. New York - Basel - Hong Kong: Marcel Dekker.
- Talieva, M.N., Kondrat'eva, V.V. & Andreev, L.N. (2001) Level of endogenous cytokinins and abscisic and salicylic acids in the leaves of *Phlox paniculata* and *Ph. setacea* under the influence of invasion by conidia of phytopathogens. *Biology Bulletin* **28**, 361-364.
- Tang, X., Rolfe, S.A. & Scholes, J.D. (1996) The effect of *Albugo candida* (white blister rust) on the photosynthetic and carbohydrate metabolism of leaves of *Arabidopsis thaliana*. *Plant Cell and Environment* **19**, 967-975.
- Tantau, H. & Dorffling, K. (1991) Effects of chilling on physiological-responses and changes in hormone levels in 2 *Euphorbia-pulcherrima* varieties with different chilling tolerance. *Journal of Plant Physiology* **138**, 734-740.
- Tarkowská, D., Doležal, K., Tarkowski, P., Āstot, C., Holub, J., Fuksová, K., Schmülling, T., Sandberg, G. & Strnad, M. (2003) Identification of new aromatic cytokinins in *Arabidopsis thaliana* and *Populus x canadensis* leaves by LC-(+)ESI-MS and capillary liquid chromatography frit-fast atom bombardment mass spectrometry. *Physiologia Plantarum* **117**, 579-590.
- Taspinar, M.S., Dumlupinar, R. & Agar, G. (2009) Determination of effects of some plant growth regulators (PGRs) on changes of some isoenzymes in bean (*Phaseolus vulgaris* L. cv. Terzibaba) at chilling temperatures: in gel enzyme assays. *Romanian Biotechnological Letters* **14**, 4858-4869.
- Thayer, S.S. & Björkman, O. (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research* **23**, 331-343.
- Thomas, J.C., Perron, M., LaRosa, P.C. & Smigocki, A.C. (2005) Cytokinin and the regulation of a tobacco metallothionein-like gene during copper stress. *Physiologia Plantarum* **123**, 262-271.
- Thomas, T.H., Hare, P.D. & van Staden, J. (1997) Phytochrome and cytokinin responses. *Plant Growth Regulation* **23**, 105-122.
- To, J.P.C., Deruère, J., Maxwell, B.B., Morris, V.F., Hutchison, C.E., Ferreira, F.J., Schaller, G.E. & Kieber, J.J. (2007) Cytokinin regulates type-A *Arabidopsis* response regulator activity and protein stability via two-component phosphorelay. *Plant Cell* **19**, 3901-3914.
- To, J.P.C., Haberer, G., Ferreira, F.J., Deruère, J., Mason, M.G., Schaller, G.E., Alonso, J.M., Ecker, J.R. & Kieber, J.J. (2004) Type-A *Arabidopsis* response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell* **16**, 658-671.
- To, J.P.C. & Kieber, J.J. (2008) Cytokinin signaling: two-components and more. *Trends in Plant Science* **13**, 85-92.
- Todorov, D., Alexieva, V. & Karanov, E. (1998) Effect of putrescine, 4-PU-30, and abscisic acid on maize plants grown under normal, drought, and rewatering conditions. *Journal of Plant Growth Regulation* **17**, 197-203.
- Todorova, D., Genkov, T., Vaseva-Gemisheva, F., Alexieva, V., Karanov, E., Smith, A. & Hall, M. (2005) Effect of temperature stress on the endogenous cytokinin content in *Arabidopsis thaliana* (L.) Heynh plants. *Acta Physiologiae Plantarum* **27**, 13-18.

- Turner, S.R., Touchell, D.H., Senaratna, T., Bunn, E., Tan, B. & Dixon, K.W. (2001) Effects of plant growth regulators on survival and recovery growth following cryopreservation. *Cryo-Letters* **22**, 163-174.
- Ueguchi, C., Koizumi, H., Suzuki, T. & Mizuno, T. (2001a) Novel family of sensor histidine kinase genes in *Arabidopsis thaliana*. *Plant and Cell Physiology* **42**, 231-235.
- Ueguchi, C., Sato, S., Kato, T. & Tabata, S. (2001b) The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in *Arabidopsis thaliana*. *Plant and Cell Physiology* **42**, 751-755.
- Van Loven, K., Beinsberger, S.E.I., Valcke, R.L.M., Van Onckelen, H.A. & Clijsters, H.M.M. (1993) Morphometric analysis of the growth of *phsp70-ipt* transgenic tobacco plants. *Journal of Experimental Botany* **44**, 1671-1678.
- Vandenbussche, F., Habricot, Y., Condiff, A.S., Maldiney, R., Van der Straeten, D. & Ahmad, M. (2007) HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in *Arabidopsis thaliana*. *Plant Journal* **49**, 428-441.
- Veselov, D.S., Sabirzhanova, I., Akhiyarova, G., Veselova, S.V., Farkhutdinov, R.G., Mustafina, A.R., Mitrichenko, A.N., Dedov, A.V., Veselov, S.Y. & Kudoyarova, G.R. (2002) The role of hormones in fast growth responses of wheat plants to osmotic and cold shocks. *Russian Journal of Plant Physiology* **49**, 513-517.
- Veselova, S.V., Farkhutdinov, R.G., Veselov, S.Y., Kudoyarova, G.R., Veselov, D.S. & Hartung, W. (2005) The effect of root cooling on hormone content, leaf conductance and root hydraulic conductivity of durum wheat seedlings (*Triticum durum* L.). *Journal of Plant Physiology* **162**, 21-26.
- Veselova, S.V., Farkhutdinov, R.G., Veselov, D.S. & Kudoyarova, G.R. (2006) Role of cytokinins in the regulation of stomatal conductance of wheat seedlings under conditions of rapidly changing local temperature. *Russian Journal of Plant Physiology* **53**, 756-761.
- Vomáčka, L. & Pospíšilová, J. (2003) Rehydration of sugar beet plants after water stress: effect of cytokinins. *Biologia Plantarum* **46**, 57-62.
- Walters, D.R. & McRoberts, N. (2006) Plants and biotrophs: a pivotal role for cytokinins? *Trends in Plant Science* **11**, 581-586.
- Walters, D.R., McRoberts, N. & Fitt, B.D.L. (2008) Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biological Reviews* **83**, 79-102.
- Wang, H.Y., Ma, L.G., Li, J.M., Zhao, H.Y. & Deng, X.W. (2001) Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* **294**, 154-158.
- Wang, Y., Yang, Z.M., Zhang, Q.F. & Li, J.L. (2009) Enhanced chilling tolerance in *Zoysia matrella* by pre-treatment with salicylic acid, calcium chloride, hydrogen peroxide or 6-benzylaminopurine. *Biologia Plantarum* **53**, 179-182.
- Waters, M.T. & Langdale, J.A. (2009) The making of a chloroplast. *EMBO Journal* **28**, 2861-2873.

- Weidhase, R.A., Lehmann, J., Kramell, H., Sembdner, G. & Parthier, B. (1987) Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methylester, and counteraction by cytokinin. *Physiologia Plantarum* **69**, 161-166.
- Werner, T., Holst, K., Pörs, Y., Guivarc'h, A., Mustroph, A., Chriqui, D., Grimm, B. & Schmölling, T. (2008) Cytokinin deficiency causes distinct changes of sink and source parameters in tobacco shoots and roots. *Journal of Experimental Botany* **59**, 2659-2672.
- Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H. & Schmölling, T. (2003) Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* **15**, 2532-2550.
- Werner, T. & Schmölling, T. (2009) Cytokinin action in plant development. *Current Opinion in Plant Biology* **12**, 527-538.
- Wilhelmová, N., Wilhelm, J., Kutík, J. & Haisel, D. (1997) Changes in French bean cotyledon composition associated with modulated life-span. *Photosynthetica* **34**, 377-391.
- Wright, D.P., Baldwin, B.C., Shephard, M.C. & Scholes, J.D. (1995a) Source-sink relationship in wheat leaves infected with powdery mildew. 2. Changes in the regulation of the Calvin cycle. *Physiological and Molecular Plant Pathology* **47**, 255-267.
- Wright, D.P., Baldwin, B.C., Shephard, M.C. & Scholes, J.D. (1995b) Source-sink relationships in wheat leaves infected with powdery mildew. 1. Alterations in carbohydrate metabolism. *Physiological and Molecular Plant Pathology* **47**, 237-253.
- Xia, J.C., Zhao, H., Liu, W.Z., Li, L.G. & He, Y.K. (2009) Role of cytokinin and salicylic acid in plant growth at low temperatures. *Plant Growth Regulation* **57**, 211-221.
- Yamaryo, Y., Kanai, D., Awai, K., Shimojima, M., Masuda, T., Shimada, H., Takamiya, K. & Ohta, H. (2003) Light and cytokinin play a co-operative role in MGDG synthesis in greening cucumber cotyledons. *Plant and Cell Physiology* **44**, 844-855.
- Yang, H.Q., Tang, R.H. & Cashmore, A.R. (2001) The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. *Plant Cell* **13**, 2573-2587.
- Yarullina, L.G., Ibragimov, R.I. & Akhmetov, R.R. (2001) Hormonal balance in wheat infected with *Helminthosporium sativum*. *Biology Bulletin* **28**, 423-425.
- Yordanov, I., Tsonev, T., Goltsev, V., MerakchiiskaNikolova, M. & Georgieva, K. (1997) Gas exchange and chlorophyll fluorescence during water and high temperature stresses and recovery. Probable protective effect of carbamide cytokinin 4-PU30. *Photosynthetica* **33**, 423-431.
- Yordanov, I., Velikova, V. & Tsonev, T. (1999) Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of bean plants. 1. Changes in chlorophyll fluorescence quenching. *Photosynthetica* **37**, 447-457.

- Yurina, T.P., Yurina, E.V., Karavaev, V.A., Solntsev, M.K., Kukushkina, M.A. & Ekobena, F.A.P. (1996) Physiological characteristics of wheat leaves in cultivars resistant and susceptible to powdery mildew. *Russian Journal of Plant Physiology* **43**, 64-69.
- Zacarias, L. & Reid, M.S. (1990) Role of growth-regulators in the senescence of *Arabidopsis thaliana* leaves. *Physiologia Plantarum* **80**, 549-554.
- Zavaleta-Mancera, H.A., Thomas, B.J., Thomas, H. & Scott, I.M. (1999) Regreening of senescent *Nicotiana* leaves: 2. Redifferentiation of plastids. *Journal of Experimental Botany* **50**, 1683-1689.
- Zažimalová, E., Kamínek, M., Březinová, A. & Motyka, V. (1999) Control of cytokinin biosynthesis and metabolism. In: *Biochemistry and Molecular Biology of Plant Hormones* (eds. P. J. J. Hooykaas, M. A. Hall & K. R. Libbenga), pp. 141-160. Amsterdam: Elsevier Science B.V.

6 Summary

PAPER I

What does trigger the cytokinin-mediated acceleration of plant senescence?

Cytokinins comprise regulators of plant growth that are well known for their ability to delay or slow down plant or leaf ageing, senescence. However, there emerge pieces of evidence that cytokinin application may cause an acceleration of senescence. We went through the literature and came to a conclusion that this reversal of cytokinin action could be connected with the application of cytokinins of high concentration and/or with the exposition of treated leaf to high light doses. The results of our experiment with detached wheat leaves treated with a cytokinin *meta*-topolin kept under continuous light or in darkness confirmed our presumption. We propose that the protective *meta*-topolin action in darkness becomes damaging in continuous light due to a feed-back inhibition of photosynthesis. *Meta*-topolin albeit preserved photosynthetic function but its coincidence with continuous irradiation led sequentially to an inordinate accumulation of assimilates, the feed-back inhibition of photosynthesis, PSII overexcitation and to induction of oxidative damage.

PAPER II

Could cytokinins serve as protecting agents against the pathogen infection?

There exist indications that cytokinin application could play a considerable role in reduction of losses caused by pathogenic invasions. Desiring to answer this question, we investigated the effect of two aromatic cytokinins; benzyladenine and *meta*-topolin, on the pathogenesis of downy mildew (*Bremia lactucae* Regel) in leaf discs of lettuce. Our results showed that cytokinins retarded *B. lactucae* development and restored host photosynthetic function; however, for successful inhibition of pathogen growth were required high concentrations of cytokinin solutions (2×10^{-4} M) and cytokinin application must have preceded the inoculation. When these high cytokinin concentrations encountered healthy tissues, after few days they induced impairment of photosynthetic apparatus similar to that caused by downy mildew infection. Thus we came to a conclusion that cytokinins probably would not be appropriate protectors against pathogen infection, at least not for lettuce endangered by downy mildew. Our results further revealed that the affect of both cytokinins and downy mildew on photosynthesis could be connected with enhanced activity of extracellular invertase. The

invertase activation, mediated by downy mildew or by applied cytokinin, led to the hexose accumulation and consequently to a feedback-inhibition of photosynthesis. This effect of cytokinins on invertase activity could at least partially explain the feed-back inhibition of photosynthesis observed previously in PAPER I. Very interesting is the fact that although both cytokinins and downy mildew acting separately induced the invertase activity (in comparison to cytokinin untreated healthy leaf discs), under their coincidence the increase was not ascertained.

PAPER III

How plants deal with low temperature strongly depends on the lipid composition of thylakoid membrane.

Only very little is known about the effects of cytokinins on plants exposed to low temperatures. Few reports demonstrated that cytokinins (either exogenously applied or endogenously elevated in *ipt*-transgenic plants) induced cold hardiness and that they might function via their influence on lipid composition of thylakoid membrane. We decided to investigate the thylakoid membrane of *Arabidopsis thaliana* wild-type and *dgd1* mutant lacking more than 90% of one of the major galactolipids of the chloroplast – digalactosyl-diacylglycerol (DGDG). The warm grown *dgd1* mutant showed markedly lower total chlorophyll and carotenoid contents and much reduced capacity for electron transport away from photosystem II. During cold acclimation, however, the *dgd1* leaves recovered the pigment-protein pools and photosynthetic function equivalent to wild-type plants. Surprisingly, this recovery was not correlated with an increase in DGDG. When returned to warm temperatures the severe *dgd1* phenotype reappeared. We conclude that the relative recovery of photosynthetic activity at 5 °C resulted from a temperature/lipid interaction enabling the stable assembly of photosystem I complexes in the thylakoid. Cytokinins were reported to take part in the formation of thylakoid galactolipids, thus now it remains for future research to establish their role during cold hardening.

7 Supplement

Following papers are attached:

- I. **Vlčková, A.**, Špundová, M., Kotabová, E., Novotný, R., Doležal, K. & Nauš, J. (2006): Protective cytokinin action switches to damaging during senescence of detached wheat leaves in continuous light. *Physiologia Plantarum* 126, 257-267.
- II. Prokopová, J., Špundová, M., Sedlářová, M., **Husičková, A.**, Novotný, R., Doležal, K., Nauš, J. & Lebeda, A. (2010): Photosynthetic responses of lettuce to downy mildew infection and cytokinin treatment. *Plant Physiology and Biochemistry*, in press, doi: 10.1016/j.plaphy.2010.04.003
- III. Hendrickson, L., **Vlčková, A.**, Selstam, E., Huner, N., Öquist, G. & Hurry, V. (2006): Cold acclimation of the Arabidopsis *dgd1* mutant results in recovery from photosystem I-limited photosynthesis. *FEBS Letters* 580, 4959-4968