L6-1: The flow of cytokinins in the environment

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It is generally accepted that cytokinins are produced in the roots and transported to the shoots. There is much evidence showing that cytokinins are transported in the xylem and phloem. Recently, there has been evidence that cytokinins are also synthesized in other parts of the plant. However, it must be questioned if all cytokinins required by the plant are made exclusively by the plant or if they can be obtained from alternative sources. We have evidence that roots are able to take up cytokinins. Different soils contain varying amounts of cytokinins depending on the plants growing in them. Maize roots release cytokinins into their growing media. Cytokinins are synthesized by soil-borne organisms such as mycorrhiza and soil microalgae. These can be released into the soil and can be extracted from soil samples. There are high concentrations of cytokinins in senescing tissues and decomposing leaves release cytokinins upon composting. Water ferns release cytokinins into water bodies. Cytokinins are also released from phloem feeding insects such as aphids. Clearly there is much potential flow and recycling of cytokinins within the environment.

O6-2: Stimulation of ribosomal RNA gene transcription in excised cotyledons of *Cucurbita pepo* L. (zucchini) in response to exogenous benzyladenine

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"Run-on" transcription has shown that treatment of excised zucchini cotyledons with BA resulted in a 2-4-fold enhancement of RNA polymerase I transcription. [^{3}H]UMP/[^{3}H]uridine ratio analysis of the alkali-digested pre-rRNA chains synthesized *in vitro* showed that this effect was due to an increased elongation rate. Size determination of the [^{32}P]CTP labeled pre-rRNA revealed that the *in vivo* effect of BA was accompanied by an increased initiation of transcription. The methylation pattern of the IGS of rRNA genes was studied using the restriction enzymes Msp I, Hpa II and Hha I and the method of "indirect end labeling" with 18S rRNA gene as DNA probe. The results showed no changes in the methylation pattern of rRNA genes in response to exogenous BA indicating that alterations in their transcription were not due to or accompanied by changes in rDNA methylation. No appreciable changes in DNAse I succeptibility of rDNA chromatin were also detected. Pretreatment with cycloheximide abolished the effect of BA, thus indicating its strong dependence on continuous protein synthesis.

O6-1: Root-synthesized cytokinin regulates root gravitropiosm and is distributed in the shoot by the transpiration stream

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In a root, cytokinin is mainly produced in the cap. From there, cytokinin is exported upward through the xylem and accumulates in the highest transpiring young shoot organs of wind-exposed Arabidopsis, whereas low or absence of free cytokinin characterizes the buds of wind-protected plants. Cytokinin has a negative regulatory role in root growth and might function as an inhibitor of tropistic root elongation during early phase of gravity response. In vertically growing Arabidopsis roots the cytokinin in the cap is distributed symmetrically. When roots are turned to a horizontal position, free cytokinin is transported laterally within less than 30 min and becomes concentrated at the new lower side of the cap, where it locally retards elongation of the lower side and promotes elongation of the upper root side. This asymmetrical activation pattern causes the initiation of a downward gravitropic root bending near the root apex. Exogenous application of cytokinin to vertical roots induced bending toward the site of application, indicating that cytokinin has an inhibitory effect in root gravitropism. Our results suggest that early root graviresponse is controlled by cytokinin. We conclude that both, cytokinin and auxin are key hormonal signals which regulate root gravitropism.

O6-3: Evaluation of the possible role of cytokinin O-glucosylation in responses of tobacco plants to drought stress

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Cytokinins (CKs) influence physiological processes (*e.g.* chlorophyll retention, stomatal functioning) affecting plant responses to stress. To determine if increased CK O-glucosylation alters drought tolerance, WT tobacco (*Nicotiana tabacum* L. cv. Wisconsin 38) and transgenic tobacco expressing a *trans*-zeatin O-glucosyltransferase (*ZOG1*) gene from *Phaseolus lunatus*, driven by a constitutive (35S) or senescence-inducible (SAG12) promoter, were compared under mild and severe drought conditions. The 35S::*ZOG1* plants showed delayed wilting during the first 2-3 days of drought. Under drought stress WT plants increased CK 7N-glucosylation, whereas transgenic plants had elevated CK O-glucosylation, particularly of *cis*-zeatin. Activity of ß-glucosidase was stimulated by drought stress in WT and transformants, especially in young leaves, and was significantly higher in 35S::*ZOG1* plants than the WT under both conditions. Transformants had substantially decreased cytokinin oxidase/dehydrogenase activity. Total photosynthetic activity decreased during drought stress but did not differ between WT and transformants. These observations indicate complex changes in cytokinin homeostasis as affected by drought stress and presence of the *ZOG1* transgene. Supported by GA CR 522/04/0549.

O6-4: The role of ABA and IAA in regulation of plant growth and adaptation under oxygen deficiency

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A comparative analysis of the effects of anoxia on growth, ABA and IAA content in wheat and rice seedlings was performed. In both plant species, a total cessation of root growth occurred during the initial hours of anoxia. Shoot elongation under the stress conditions was estimated only in rice seedlings. During the initial hours of anoxia, the level of free ABA in wheat and rice tissues increased manifold, and the accumulation of a free ABA form occurred at the expense of the hydrolysis of its bound forms. The IAA content in plant tissues also increased. In wheat, the accumulation of IAA was short, but in rice, a high hormone level was retained during the entire experiment, and, as a result, its concentration exceeded that of ABA. It is suggested that IAA accumulation in hypoxia-tolerant rice seedlings under anoxia favors maintenance of shoot growth and simultaneous inhibition of root growth. At the same time, in the hypoxia-sensitive wheat, an increase in the ABA level resulted in growth cessation. A treatment with exogenous ABA and IAA before anoxia action resulted in elevated seedling survival measured by tetrazolium test and decreased lipid peroxidation during post-anoxic stress.

O6-6: Some observations of potato plants overexpressing an *ipt* gene: leaf physiology and the response to CO_2 enrichment

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With higher levels of Z-type cytokinins, the *ipt*-potato plants showed higher rates of photosynthesis, transpiration and stomatal conductance per unit leaf area than the wild-type (WT) plants under favourable growth conditions (high humidity, moderate light levels) in normal ambient [CO_2] (360 ppm). Analyses of leaf tissues showed that the *ipt*-plants have higher leaf nitrogen (43 %) and chlorophyll (62 %), less negative $d^{13}C$ values (1.2 ‰), and lower $d^{18}O$ values (2.3 ‰) than the WT plants. The isotopic composition of leaf dry matter and gas exchange measurements indicated that *ipt*-potato plants have significantly higher stomatal conductance and greater photosynthetic capacity. The greater photosynthetic capacity of transgenic leaves may be due to the production and development of more chloroplasts by increased CK levels. Growth of WT and *ipt*-potato plants in high nitrogen nutrition (12 mM) was stimulated by elevated [CO_2] (EC; 900 ppm). Growth enhancement in EC was observed for both WT and *ipt*-potato leaves, stems, tuber mass, and tuber number. Growth in EC, however, caused a significant decrease in tuber nitrogen content (14.5 %) and a small decease (9 %) in leaf chlorophyll content in WT and *ipt*-potato plants.

O6-5: IAA biosynthesis in *Rhizobia* and its potential role in symbiosis

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A role for plant hormones in nodulation and the effect of the Nod-factor on the hormone balance in the host is frequently reported in literature. Rhizobia are able to produce indole-3-acetic acid and host derived flavonoids can increase the bacterial IAA synthesis. NGR234 shows both a constitutive and a flavonoid induced IAA biosynthesis. This flavonoid induced IAA synthesis is NodD1, NodD2 and SyrM2 dependent and the gene cluster y4wEFG, encoding this IAA pathway, is located downstream a Nodbox (Nodbox 15) (Theunis et al., MPMI17:1153-1161, 2004). Knockout mutations in y4wE and y4wF abolished flavonoid-inducible IAA synthesis and a functional y4wF is also required for constitutive IAA production. Analysing indole-intermediates, show y4wE and y4wF encoding a Trp-amino transferase and an IAAld oxidase respectively, both encoding critical steps in the IPyA pathway. The effect of an impaired flavonoid induced IAA synthesis in NGR234 is host dependant. A hypothesis on the role of the bacterial versus host derived auxin will be discussed.

P6-1: Cytokinin content in relation to MeJA and dark-induced senescence in intact cotyledons of *Cucurbita pepo* L. (zucchini)

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A study on the senescence promoting effects of methyl ester of jasmonic acid (MeJA) and darkness in *Cucurbita pepo* cotyledons was carried out. Seven-day-old seedlings were either sprayed with MeJA (100 µM) or transferred to darkness. Both effectors induced 24 h after the treatments senescence as judged by the decrease in chlorophyll content. Chlorophyll fluorescence and the rate of photosynthesis measured by PAM-fluorimeter and LiCor 6400, respectively, were also decreased. MeJA affected all these parameters to a higher extent as compared to darkness. Transcription *in organello* with isolated intact chloroplasts showed an inhibition of [^{3}H]-UTP incorporation in total chloroplast RNA - the jasmonate effect being 2-fold higher than darkness. The data on senescence parameters correlated with lowered endogenous levels of physiologicaly active cytokinins (CK) determined by HPLC-MS. Levels of *trans*-zeatin, dihydrozeatin, isopentenyladenine and their ribosides decreased after dark and MeJA treatment by 25% and 53%, respectively, as compared to the control. These results suggest that MeJA-mediated senescence in zucchini cotyledons was much more pronounced when compared to darkness, most probalby due to its stronger downregulation effect on endogenous cytokinins.

P6-2: Effect of prolonged exposure to salinity on the major cytokinins in pea plants

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Trans- and *cis*-zeatin (Z), N^6-isopentenyladenine (iPA), dihydrozeatin, and their ribosides, mono-phosphates and O-glucosides were the major cytokinins (CKs) identified in pea plants (*Pisum sativum*, cv. Ran). CKs levels differed between plant organs and organ parts. Growth and development of pea plants were much affected by long-term exposure to salinity (150 mM NaCl): root and shoot size and fresh mass were reduced by a half, the time to flowering was shortened. Lower levels of iPA-type ribosides and mono-phosphates in roots and increased levels of Z-type ribosides in the whole plant were found in response to salinity. (Supported by NCSR B-1208 and by GACR 522/04/0549.)

P6-3: Temperature variances modulate cytokinin content and cytokinin oxidase/dehydrogenase activity in young pea plants

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Cytokinins (CKs) play an important role in several aspects of plant growth, metabolism and development at normal growth conditions. The mechanism by which environmental changes affect CK levels and cytokinin oxidase (CKX) activity (EC 1.5.99.12) is still not clear. Mild abnormal temperatures (10º and 33ºC average) were applied on young pea plants from two varieties (cvs. Manuela and Scinado) in order to assess CK pool and CKX activity response towards altered growth conditions. Both temperature treatments increased iP/iPR content in leaves and roots. This trend was dramatically exhibited in leaves and cold treatment additionally resulted in elevated *cis*ZR content and CKX activity. High temperature did not influence enzymatic activity in leaves. In roots of the two varieties opposite trends in CKX response towards temperature treatments were observed – Manuela roots had lower CKX while Scinado responded to adverse temperatures with increased enzymatic activity. Data suggest that variance in temperatures provokes adaptive reactions in cytokinin pool which may trigger changes in enzymatic activities participating in hormonal metabolism. CKX response toward low and high temperature varies in different cultivars and exhibits certain tissue specificity.

P6-4: Treatment with cytokinins delays chlorophyll degradation induced by *Bremia lactucae* pathogenesis on host *Lactuca spp*.

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Cytokinins (CKs), as an important group of plant growth regulatory substances, affect a wide array of biological processes. They also can delay leaf senescence caused by both internal and external factors. CKs as senescence antagonists can prevent peroxidation of plasma membrane, degradation of chlorophyll, favour chloroplast differentiation, *etc*. Infection by fungal pathogens may elevate endogenous level of CKs and some biotrophs produce CKs themselves to alter plant response to infection. In contrary, exogenous treatment with CKs eliminates powdery mildew development on compatible genotypes of crops or elevates plant tolerance to necrotrophs. Action of CKs is specific for each pathosystem and their effects are dose-dependent. In *Lactuca* spp. genotypes susceptible to *Bremia lactucae* severe infection and sporulation intensity of pathogen was suppressed by 24-hours pre-incubation with aromatic CKs (m-topolin, MeOBAPR) in concentration $2x10^{-4}$ M. Increased resistance to causal agent of lettuce downy mildew in treated leaf segments was during 14days of incubation followed by delayed senescence and retention of chlorophyll, which has been known for senescence caused by heat stress and drought. (The work is supported by GAČR 522/02/D011, MSM 6198959215.)