NATURAL VARIATION IN THE CYTOKININ METABOLIC NETWORK IN *ARABIDOPSIS THALIANA*

PŘÍRODNÍ VARIACE METABOLISMU CYTOKININŮ U *ARABIDOPSIS THALIANA*

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ABSTRACT

Cytokinins are key determinants of cellular plasticity and are crucial for several processes that together determine overall fitness under any given conditions.

Like any other signal molecule, the action of cytokinins is determined by the amount of active hormone exposed to receptor molecules, and the strength and duration of the subsequent signalling. Active hormone levels are the result of the combinatorial regulation of their biosynthesis, reversible and irreversible metabolic interconversion, and terminal degradation.

The existence of genetically determined variation in geographically distinct populations within the model species *Arabidopsis thaliana* is being increasingly documented. We are exploring the cytokinin status of young Arabidopsis seedlings from a collection of accessions that represent maximal genetic diversity within the species. We will present data regarding cytokinin levels and some examples of physiological analysis of the variation in response to exogenous cytokinin. Comprehensive mapping of the variation in cytokinin metabolism will allow us to establish a basis for understanding how relatively minor changes in hormone status are used by the plant to form and fine-tune adaptive processes.

Key words: *Arabidopsis thaliana*; cytokinin metabolism; homeostasis; metabolic and regulatory networks; natural variation; zeatin

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INTRODUCTION

Tightly regulated cellular plasticity is the main response mechanism in plants to changing environmental conditions. Phytohormones have simple chemical structures, but are capable – on their own and in co-operation with each other – of bringing about profound changes in plant structure and physiology. Naturally occurring cytokinins (CKs) are N^6-substituted adenine derivatives and are involved in regulating a wide range of developmental processes including chloroplast differentiation, nutrient assimilation and translocation, seed germination, leaf expansion, flowering, and senescence (reviewed in Sakakibara 2006). Highly active molecules like CKs have a rather narrow band of concentrations of the active form at the steady-state and this has been confirmed via quantification in several plant species (Benková et al., 1999, Kiran et al., 2006, Hradilová 2007). This steady state is the result of a combinatorial regulation of all the processes that the molecule is subject to, including, among others, biosynthesis, transport, conjugation and degradation. The resulting level at which this steady-state is established can vary depending on the plant species (monocot/dicot), plant part (cell/tissue/organ), developmental phase (flower bud/developing fruit), internal cues (other hormones/nutrition status), external cues (light/pathogen), etc. We have previously determined that the plant probably uses modification of zeatin metabolism to vary internal CK levels even when the external source of CKs is of the non-zeatin type (Lexa et al., 2003). Thus the regulation of zeatin-type CKs is a central component of overall CK metabolism *in planta*.

There is growing interest in the response of naturally occurring Arabidopsis accessions and a variety of abiotic and biotic factors, and these investigations have resulted in the recognition that natural Arabidopsis diversity can be used to discover information about those factors. The existence of natural variation in pathways that are crucial for the co-ordination of developmental and environmental cues is becoming increasingly clear form various recent studies focused on analyzing diversity among Arabidopsis accessions for a variety of physiological processes such as nitrogen uptake (Chardon et al., 2010), response to the hormone salicylic acid (van Leeuwen et al, 2007) and resistance to the biotrophic pathogen *Botrytis cinerea* (Rowe & Kliebenstein, 2008). Therefore, *A. thaliana* natural variation provides a relevant resource to uncover such gene functions, particularly for traits related to adaptive plasticity, such as those controlling CK homeostasis. Analyzing the existing variation among different *A. thaliana* accessions with regard to accumulation patterns of different CK metabolites is expected to yield information about the regulatory mechanism(s) controlling CK homeostasis.

MATERIALS AND METHODS

Seeds of 20 Arabidopsis accessions described previously as representing maximal genetic diversity in the species (Clark et al., 2007) were obtained from the Nottingham Arabidopsis Stock Centre. Seeds were sterilized, plated on square petri plates containing 0.5X MS medium, without sucrose and solidified with 1.2% agar. The seeds were stratified in the dark at 4 ˚C for 3 days and then transferred to a cultivation chamber (Percival AR36L, Percival, USA) and incubated under long day conditions (16h light; 21 ˚C/8h dark; 19 ˚C) with illumination corresponding to 90-100 μmol photons m^{-2} s^{-1}. Seedlings were harvested at 14 and 21 days after sowing (DAS) with the roots and shoots collected separately. The samples were then frozen and stored until processed for CK quantification. CK extraction and quantification was carried out in the Laboratory of Growth Regulators, Palacký University according to established procedures (see e.g. Novák et al. 2008). The root inhibition tests were carried out under identical conditions with the exception that the test medium was supplemented with 1 μM trans-zeatin. These seedlings were photographed at 6, 8, 10, 12 and 14 DAS and root lengths measured using ImageJ software (http://rsb.info.nih.gov/ij/). The hypocotyl elongation test was conducted under similar conditions except that the light intensity was 20 μmol photons m^{-2} s^{-1} and the seedlings were photographed at 11 DAS.

RESULTS AND DISCUSSION

Arabidopsis accessions show a distinct variation in CK metabolite levels

The collection of 20 genetically diverse Arabidopsis accessions show distinct variations in CK levels. We measured the levels of all isoprenoid CK derivatives in the roots and shoots separately at 14 DAS and 21 DAS and we found that the total CK levels showed substantial differences when compared to the reference accession Col-0 (Fig. 1). Col-0 has roughly the same levels of iP-type and zeatin-type CKs, as do some other accessions, like NFA-8, RRS-7, Bay-0 and C24. However, we have identified accessions that are significantly enriched in zeatin-type CKs like Tsu-1, Bor-4 and Van-0 (Fig 1). Some accessions like Br-0, Got-7, Bur-0 and Ts-1 display the reverse, i. e., are significanlty enriched in iP-type CKs (Fig.1). It is already known that the iP-type CKs are probably synthesised by AtIPT7 and AtIPT8 (Miyawaki et al., 2004; Sun et al., 2003). On the other hand zeatin-type CKs are probably synthesised in the chloroplasts by the action of enzymes like AtIPT3 (Kasahara et al.,

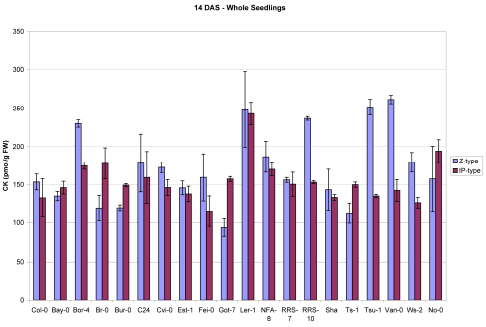

Figure 1: Quantification of isoprenoid CKs from whole 14-day old seedlings, blue bars – zeatin-type and red bars – iP-type CKs.

2004). Thus we predict that there could be widely different regulation of these gene products in our collection of accessions. A comprehensive analysis of the transcriptional regulation of these genes is a challenge for the future and will form part of a separate study.

Organ-specific distribution of CK levels

The analysis of CK levels has also revealed a substantial difference between the root and the shoot in terms of the steady-state levels of CK metabolites. We find that in general the roots have significantly higher levels of CKs in comparison to the shoots and that this difference is true for all the accessions in our collection. A similar distribution is found at both 14 and 21 DAS. That CKs play opposite roles in the root and the shoot has been shown by over-expression of CK-degrading enzymes (see e.g., Werner et al., 2008). Our results with the diverse accessions point to a more general trend which probably led to the conclusion (before the discovery of plant-encoded IPTs) that CKs were produced exclusively in the roots (see e.g., Letham & Palni, 1983). Our observations lends credence to the hypothesis that the root is the predominant organ of CK porduction. However this then begs the transport question and the differential distribution of CK types in the xylem and phloem sap (Kudo et al., 2010) needs to be fully explained.

Response to exogenous CK is different

We have established that Arabidopsis seedlings elongate their hypocotyls in response to exogenous zeatin when grown under low light intensity (20 µmol m^{-2} s^{-1}). In this bioassay we have found that there is considerable variation in the physiological response of the hypocotyl to zeatin (Fig. 2).

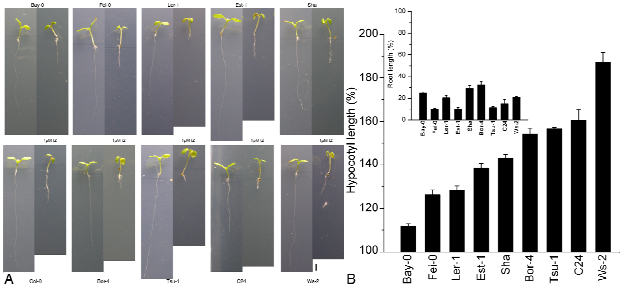

Figure 2: CK-dependent stimulation of hypocotyl elongation in Arabidopsis accessions under decreased light intensity. A: photographs of 11-day-old seedlings on medium with (right) and without (left) 1µM tZ. B: quantification of results from A, expressed as percentages of the respective untreated controls.

Under normal light intensities, preliminary results from the root elongation bioassay shows that there is probably a substantial difference in the sensitivities of these accessions to exogenous zeatin (Fig. 3). The sensitivity of the plant to CKs is determined by the activity and the sensitivity of the different CK receptors. Recent work has shown that the sensitivity of the plant ot CKs is determined by the expression and distribution of CK receptors that have different substrate specificities (Stolz et al., 2011). A thorough examination of the CK receptor expression profile enabling us to explain this divergent response to exogenous CK will be the subject of future research.

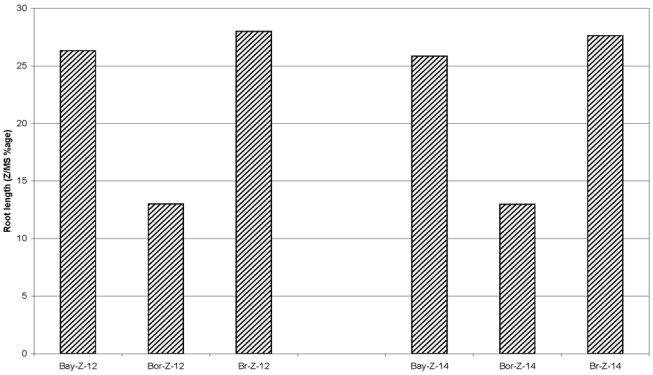

Figure 3: CK-dependent inhibition of root elongation is significantly different in Bor-4 from two other accessions at both 12DAS (left) and at 14DAS (right).

CONCLUSIONS

We have determined the metabolite profile of isoprenoid CKs in a collection of Arabidopsis accessions representing maximal genetic diversity.

The CK profiles show a substantial variation in iP- and zeatin-type CKs.

We observed a general trend of higher total CK content in the roots.

Preliminary experiments have produced results that show substantial differences in the sensitivities to exogenous CKs in two different bioassays.

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