

Transnational Access Report

1. General Information

Project Acronym (ID):	CytDroug2
Project Title	Elucidation of the functions of the plant hormones cytokinins during the drought stress response in Arabidopsis (second part)
Installation used	SCREEN-Chamber
Name of Group Leader	Radomira Vankova
Name of organization	Institute of Experimental Botany AS CR

2. Duration of access

Begin of the project	End of the project
2.3.2015	29.4.2015

3. Project summary (max. 250 words)

The effect of modulation of cytokinin (CK) levels on the *Arabidopsis thaliana* drought response and recovery was followed. The effect of CK down-regulation was studied using transformants over-expressing gene for the main CK deactivating enzyme cytokinin oxidase/dehydrogenase (*CKX*), either constitutively (35S:CKX1) or at the stress onset (pOp/LhGR:CKX). The impact of CK up-regulation was followed using transformants over-expressing gene for the rate limiting CK biosynthetic enzyme isopentenyltransferase (*IPT*) stimulated at the stress onset (pOp/LhGR: *IPT*) or during the stress progression (SAG12:*IPT*). Transgenic approach was combined with the pharmacological one by application of exogenous CK m-topolin. The levels of 24 CK metabolites were determined before the stress initiation, in the end of drought period and after 7-day recovery using LC/MS/MS. Taking into account phytohormone cross-talk, also other hormones were determined, i.e. indole-3-acetic acid, abscisic acid, salicylic acid and jasmonic acid (including their metabolites). The expression levels of selected CK- and abscisic acid-related genes as well as stress-marker genes (*RD29B* and *PCS1*) were determined by quantitative RT PCR. Phenotyping analysis (determination of rosette diameter, rosette area and photosynthetic parameters) allowed characterization of the physiological performance of the individual experimental variants. CK down-regulation significantly enhanced stress tolerance, but allowed only limited re-growth during recovery. CK up-regulation (especially in case of SAG:*IPT*) diminished stress impact to lower extent in comparison with *CKX* over-expressers, however, it had strong positive effect on plant growth stimulation during recovery.

4. Main achievements (max. 250 words)

This phenotyping and hormonal study allowed characterization of the impact of modulation of cytokinin (CK) levels on *Arabidopsis thaliana* drought response and recovery. Drought imposed in all genotypes (with exception of activated *IPT* transformants) decrease of active CKs, predominantly of those associated with stimulation of cell division – *trans*-zeatin and its riboside. Slowly growing *CKX* transformants exhibited relatively mild CK decrease, associated with only minor stimulation of drought stress marker gene expression. The enhanced stress defense, however, was maintained for some time after re-watering, which negatively affected their re-growth. The *IPT* transformants exhibited upon stimulation high levels of all CK metabolites (which indicated plant tendency to reduce CK content at water limitation conditions). Enhanced CK content had positive effect especially during recovery, when the growth rate exceeded

considerably that of the control, well-watered plants. Drought induced growth suppression was associated also with decrease of the other cell division promoting hormone – auxin (to lower extent in IPT transformants). CKX over-expressers had lower indole-3-acetic acid levels (under all conditions) in comparison with the other experimental variants. The most general drought response was elevation of abscisic acid, comparable (in drought) in all genotypes. Drought induced also increase of jasmonic acid and its active metabolite jasmonate-isoleucine, especially in Col-0. Lower, but still highly significant elevation was observed after application of exogenous CK or after IPT stimulation. It is not clear whether low jasmonic acid levels in CKX transformants are related to their higher stress tolerance. No consistent response was found in case of salicylic acid.

5. Publications related to the access granted, acknowledging the support by EC.

Poster: S. Prerostova, P. I. Dobrev, N. Koerber, R. Pieruschka, B. Brzobohaty, M. Cerny, L. Spichal, A. Gaudinova, V. Knirsch, F. Fiorani, R. Vankova: Elucidation of the functions of the plant hormones cytokinins during the drought stress response in Arabidopsis; In: EPPN Plant Phenotyping Symposium, 2015, Abstract Book (11.-12.11.2015, Barcelona, Spain, p.32)

Sylva Prerostova, Petre I. Dobrev, Niklas Koerber, Roland Pieruschka, Bretislav Brzobohaty, Martin, Cerny, Lukas Spichal, Alena Gaudinova, Vojtech Knirsch, Fabio Fiorani, Radomira Vankova: Characterization of the functions of the plant hormones cytokinins during the drought stress response in Arabidopsis (manuscript in preparation)