

Transnational Access Report

1. General Information

Project Acronym (ID):	CytDrought
Project Title	Elucidation of the functions of the plant hormones cytokinins during the drought stress response in Arabidopsis
Name of Group Leader	Radomira Vankova
Name of organization	Institute of Experimental Botany AS CR

2. Access to the Installation

ScreenChamber

3. Project summary (max. 250 words)

The experiment started on March 3 when seeds of wild type Col0, another wild-type routinely used in the host laboratory as well as majority of transformants [root-targeted (*PYK10:CKX3*) or chemically induced (*pOp/LhGR:CKX*) *CKX*-over-expressors and dexamethasone (*pOp/LhGR:IPT*) or senescence-inducible (*SAG:IPT*) *IPT*-over-expressors] were sown. The slowly growing constitutive *CKX* over-expressor (*35S:CKX1*) was sown already on February 17. After 16 days (March 19), phenotypic measurements, including the determination of leaf area, stockiness and photosynthetic parameters, started. Induction of the expression of *CKX* and *IPT*, respectively, was done by spraying of the respective transformants with dexamethasone (20 μ M, in DMSO). Simultaneously, exogenous aromatic *CK* (10 μ M) and *CKX* inhibitor Incyde (10 μ M) were sprayed on Col0 as additional experimental variants. The spraying occurred on March 28, April 4 and April 10. Drought was imposed on March 29 by withdrawal of water to half of the plants. Water content of well-watered, control trays was maintained by replacing any water lost. Drought was terminated on April 11. Then the recovery response was followed. The samples for hormone analyses were collected before the stress initiation (March 28), at the end of the drought period (April 11) and at the end of recovery (April 15). The hormones were extracted with modified Bielecki solution, purified by reverse phase chromatography and separated by ion exchange chromatography. Hormone metabolites (63) were determined by LC/MS. Expression of introduced genes was verified by quantitative RT PCR (samples taken at the end of the experiment).

4. Main achievements (max. 250 words)

Phytohormone analysis showed strong negative effect of drought on the content of active cytokinins (*CKs*) and their precursors in all experimental variants (with exception of *SAG:IPT*). Simultaneously, suppression of the growth rate was observed, minor in case of slowly growing *CKX* transformants and after exogenous *CK* application. The over-expression of *CKX* was associated with decreased basal levels of abscisic acid. Drought stress resulted in strong up-regulation of this stress hormone in all variants. Lower *CK* levels in *CKX* transformants coincided with lower levels of auxin (indole-3-acetic acid). Drought-induced decrease of auxin levels was observed in Col0 and *PYK10:CKX* plants. Preservation of auxin levels in strong *CKX* and *IPT*-over-expressors might indicate their enhanced drought tolerance. Prolonged drought was associated in all variants with elevation of another stress hormone - salicylic acid, less pronounced in constitutive *CKX* transformant and in *SAG:IPT* plants. Elevation of jasmonic acid as well as jasmonate isoleucine was observed in all *CKX* transformants, while its down-regulation was found in *IPT* transformants, at the presence of exogenous *CK* and after application of Incyde. Efficiency of photosystem II (F_v/F_m) slightly decreased during the drought stress in all variants.

Recovery phase was associated with strong up-regulation of growth rate as well as concentration of active CKs, to higher extent than in control plants. Simultaneously, relative water content of recovered plants was higher than that of the corresponding controls. The exception were 35S:CKX plants, probably due to their changed leaf morphology. The achieved results revealed the characteristic hormonal responses to drought.

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

Please specify the type of publication or presentation (scientific journal, book, patent, abstract, proceedings, article) and provide the full reference or link.

The achieved results represent a solid basis for article in impacted journal.