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## Effects of elevated endogenous cytokinin levels on the *Arabidopsis thaliana* proteome

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## I. Introduction

The plant hormone cytokinin (CK) plays a crucial role in plant development from germination to senescence, being thus a subject of intense research since its discovery. Sequencing of the genome of the model plant *Arabidopsis thaliana* facilitated global approaches to the study of CK action in plant development. We report here a classical proteome-based approach as an attempt to reveal more information about CK action in *Arabidopsis*. The availability of an improved pOp-*ipt*/LhGR transgenic system in which the production of CK is inducible by dexamethasone (DEX) (Craft et al., 2005) allowed us to compare proteomes of plants with large differences in endogenous CK content. To exclude the potential effects of DEX on protein composition, wild type Columbia-0 was included in the experiment as a control.

## II. Results

### 1. Plant material

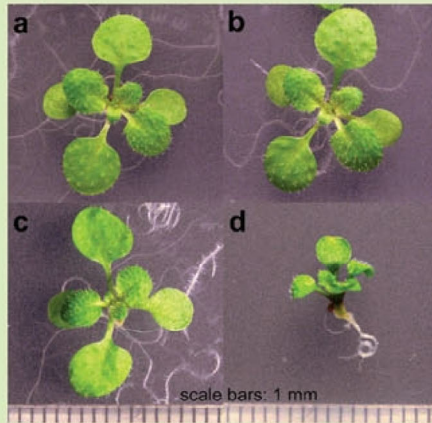


Fig. 1: Seedlings of wild-type Columbia 0 (upper row) and a transgenic pOp-*ipt*/LhGR line (lower row) were grown for 15 days on MS medium in the absence (a,c) and presence (b,d) of 2.5 μM DEX, under following cultivation conditions: 16h light/8h dark; 21 °C day/19 °C night; light intensity 117 μmol.m<sup>-2</sup>.s<sup>-1</sup>. Whole plantlets were harvested and frozen in liquid nitrogen, followed by extraction of total proteins and CKs from parallel samples.

### 2. Quantitative analysis of cytokinins

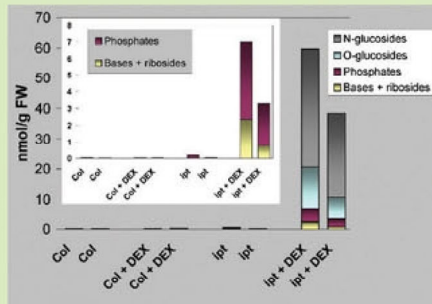


Fig. 2: CKs were extracted by methanol/chloroform/acetic acid and purified on reversed-phase C18 columns. Quantification was performed by LC/MS using deuterated CKs as internal standards. All samples (marked Col, Col + DEX, ipt and ipt + DEX; corresponding to a, b, c and d in Fig. 1, respectively) were analysed in duplicates.

### 3. Two-dimensional electrophoresis

For the first dimension, 18 cm IPG strips pH 4-7 were loaded with 500 μg protein. The second dimension, SDS-PAGE, was performed on a 11% polyacrylamide gel in a classical set-up. Gels were stained with colloidal Coomassie Brilliant Blue G250. Image analysis of two-dimensional gels in triplicates and subsequent statistical evaluation revealed significant differences between protein maps from DEX treated and non-treated pOp-*ipt*/LhGR plants (Fig. 3), whereas no significant effects of DEX on the protein maps from wild-type plants were observed. The parameters for considering significant a change in spot intensity were the following: normalised spot volume 0.050 and higher,  $p < 0.05$  at Student's t-test, well defined and bordered spot.

### Acknowledgements

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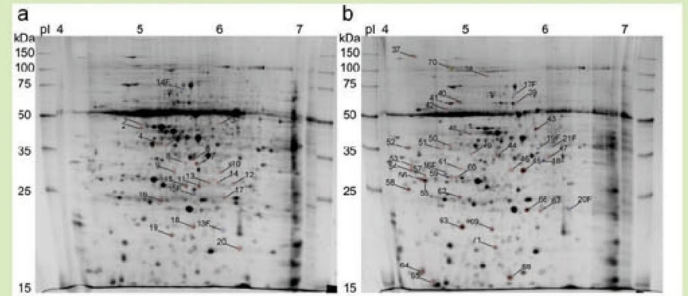


Fig. 3: Protein maps of pOp-*ipt*/LhGR plants grown in the absence (a) and presence (b) of 2.5 μM DEX. The numbers indicate the spots that were significantly overexpressed in the respective sample and analysed by MALDI-TOF MS.

### 4. Protein identification and functional classification

Total of 62 differentially expressed spots matching the above mentioned parameters were picked, in-gel digested with trypsin and subjected to MALDI-TOF MS analysis; 58 of them were characterized: 2 specific, 35 upregulated, 19 downregulated and 2 absent in gels from DEX treated pOp-*ipt*/LhGR plants (see abbreviated list in Table 1). The identified proteins were classified into functional categories (Fig. 4).

Table 1: List of selected differentially expressed proteins in DEX induced (compared to non-induced) pOp-*ipt*/LhGR plants.

| Regulation <sup>a</sup> | p <sup>b</sup> | Spot <sup>c</sup> | Protein <sup>d</sup>                                   | Accession No. <sup>e</sup> | MOWSE score <sup>f</sup> | Seq. cover. <sup>g</sup> |
|-------------------------|----------------|-------------------|--|----------------------------|--------------------------|--------------------------|
| Up                      | 0.001          | 45                | Jasmonate inducible protein isolog                     | O04314                     | 286                      | 67%                      |
|                         | 0.01           | 18F               | 2'-hydroxyisoflavone reductase                         | P52577                     | 112                      | 37%                      |
|                         | 0.01           | 37                | Cor78 protein  | Q39060                     | 273                      | 35%                      |
|                         | 0.05           | 20F               | Glutathione-S-transferase                              | P42760                     | 115                      | 46%                      |
|                         | 0.05           | 43                | S-adenosylmethionine synthase                          | Q9LUT2                     | 338                      | 67%                      |
|                         | 0.05           | 61                | Phosphoribosylformylglycinamide cyclo-ligase precursor | Q05728                     | 115                      | 20%                      |
|                         | 0.05           | 66                | GSH-dependent dehydroascorbate reductase               | Q9FWR4                     | 269                      | 83%                      |
| Down                    | 0.01           | 13                | L-ascorbate peroxidase                                 | Q8STM9                     | 231                      | 50%                      |
|                         | 0.05           | 10                | Probable ethylene-inducible protein                    | O80448                     | 207                      | 46%                      |

Each entry contains: <sup>a</sup>Type of regulation by DEX induction; <sup>b</sup>Significance of quantitative change; <sup>c</sup>Spot number referring to that in Fig. 3; <sup>d</sup>Best matching result of Mascot search; <sup>e</sup>Swiss-Prot accession number; <sup>f</sup>Significance of protein identification ( $p < 0.05$ ; cut-off level = 65); <sup>g</sup>Percentage of protein sequence covered by MALDI-TOF MS analysis.

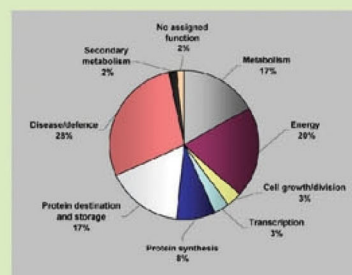


Fig. 4: Assignment of the differentially expressed proteins to functional categories using the classification described by Bevan et al. (1998).

## III. Conclusions

- The extremely high endogenous cytokinin content in DEX induced pOp-*ipt*/LhGR plants corresponds to the high number of proteome changes expected and detected. On the other hand, no major effects of DEX were observed when applied to wild-type plants.
- Two-dimensional electrophoresis as a top-down approach is a suitable tool for identifying candidates related to CK action on the protein level though it can never cover the whole protein spectrum.
- We have identified several proteins known as related to CK (e. g. S-adenosylmethionine synthase, an enzyme of the ethylene biosynthesis pathway) as well as proteins whose link to CK was not shown previously.
- The high CK levels in DEX induced pOp-*ipt*/LhGR plants seem to affect (probably indirectly) the expression of numerous proteins previously known as disease/stress responsive.

### References:

- Bevan M, et al. (1998) *Nature* 391:485-488  
 Craft J, et al. (2005) *Plant J* 41:899-918