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# LINK BETWEEN CYTOKININS, OXIDATIVE STRESS AND JASMONIC ACID AS REVEALED BY PROTEOME ANALYSIS IN *ARABIDOPSIS*

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## I. Introduction

Cytokinins (CKs) regulate diverse developmental processes in plants. The molecular mechanisms of CK action are currently the subject of intense research. We analyzed changes in the *Arabidopsis* proteome caused by increase in endogenous CKs which was achieved by the employment of an improved pOp*-ipt*/LhGR transgenic system in which the production of CKs is inducible by dexamethasone (DEX) (Craft et al., 2005).

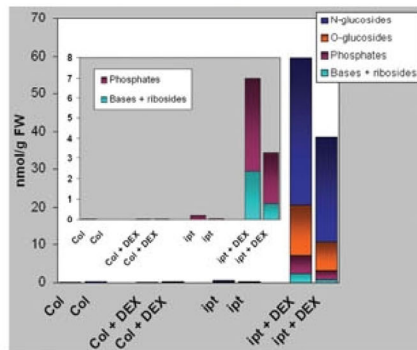
## II. Results

### 1. Plant material



**Fig. 1:** Wild-type Columbia 0 (Col; control) and a transgenic pOp*-ipt*/LhGR line (ipt) grown for 15 days on MS medium in the absence and presence of 2.5 mM DEX, at 16h light/8h dark; 21 °C day/19 °C night; light intensity 117 mmol.m<sup>-2</sup>.s<sup>-1</sup>.

### 2. Quantitative analysis of CKs

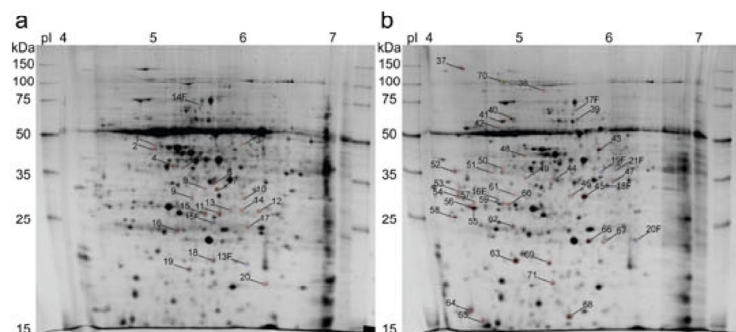


**Fig. 2:** CKs extracted by methanol/chloroform/acetic acid and purified on RP-C18 columns were quantified by LC/MS using deuterated CKs as internal standards. Samples were analysed in duplicates.

### 3. Two-dimensional (2-D) electrophoresis

2-D electrophoresis was performed in a classical set-up (IPG strips pH 4-7; 500 mg protein/strip; 11% SDS-PA gel;

colloidal CBB-G250 staining). Analysis of gel images in triplicates and subsequent statistical evaluation revealed significant differences between protein maps from DEX treated and non-treated pOp*-ipt*/LhGR plants (Fig. 3). No significant effects of DEX on wild-type protein maps were observed



**Fig. 3:** Protein maps of pOp*-ipt*/LhGR plants grown in the absence (a) and presence (b) of 2.5 mM DEX; numbers indicate the significantly overexpressed spots.

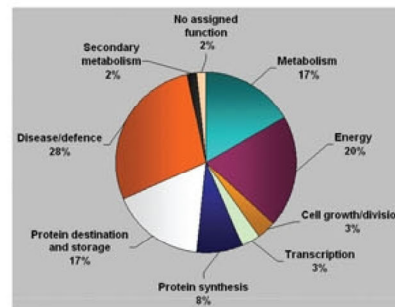
### 4. Protein identification and functional classification

Total of 62 differentially expressed spots were 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 (Table 1); and classified into functional categories (Fig. 4).

**Table 1:** Abbreviated list of differentially expressed proteins

| 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                     | 266                      | 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                          | Q8LUT2                     | 338                      | 67%                     |
|                         | 0.05           | 61                | Phosphoribosylformylglycinamide cyclo-ligase precursor | Q05728                     | 115                      | 20%                     |
|                         | 0.05           | 66                | GSH-dependent dehydroascorbate reductase               | Q9FWR4                     | 289                      | 83%                     |
| Down                    | 0.01           | 13                | L-ascorbate peroxidase                                 | Q8STM9                     | 231                      | 50%                     |
|                         | 0.05           | 10                | Probable ethylene-inducible protein                    | O80448                     | 207                      | 46%                     |

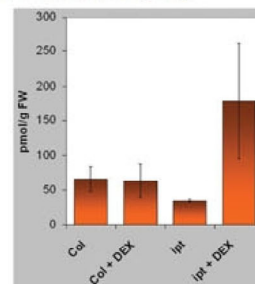
Each entry contains: <sup>a</sup>Type of regulation by DEX induction; <sup>b</sup>Significance of quantitative change; <sup>c</sup>Spot number referring to that in Fig. 3; <sup>d</sup>Best matching result of Mascot search; <sup>e</sup>Swiss-Prot accession number; <sup>f</sup>Significance of protein identification (p < 0.05, cut-off level = 85); <sup>g</sup>Percentage of protein sequence covered by MALDI-TOF MS analysis.



**Fig. 4:** Assignment of the differentially expressed proteins to functional categories according to Bevan et al. (1998).

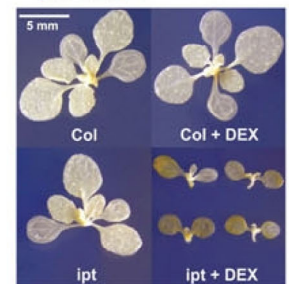
Validity of results from 2-D analysis such as the high proportion of defence/oxidative stress related proteins in induced plants was further examined by the following experiments:

### 5. Quantitative analysis of jasmonic acid (JA)



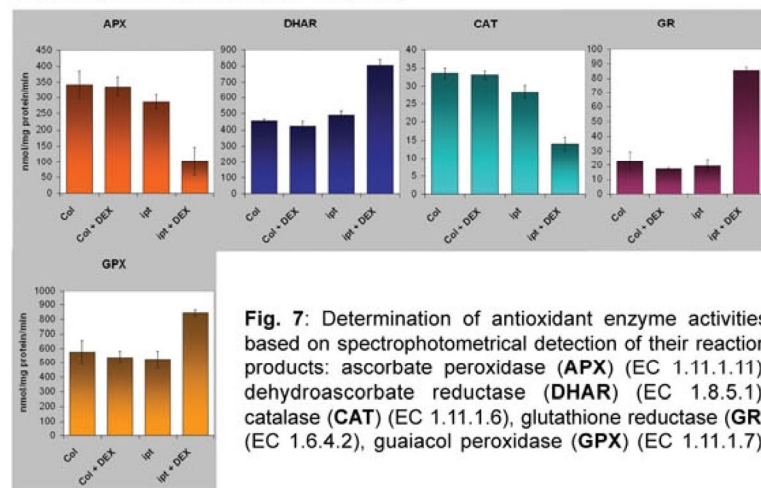
**Fig. 5:** Content of JA as quantified by LC/MS; samples were analyzed in triplicates.

### 6. Localization of hydrogen peroxide



**Fig. 6:** Chromogenic detection of H<sub>2</sub>O<sub>2</sub> in plant tissues by diaminobenzidine (DAB) staining (2.5 mM/2 h).

### 7. Assays for antioxidant enzymes



**Fig. 7:** Determination of antioxidant enzyme activities based on spectrophotometrical detection of their reaction products: ascorbate peroxidase (APX) (EC 1.11.1.11), dehydroascorbate reductase (DHAR) (EC 1.8.5.1), catalase (CAT) (EC 1.11.1.6), glutathione reductase (GR) (EC 1.6.4.2), guaiacol peroxidase (GPX) (EC 1.11.1.7).

## III. Conclusions

- Image analysis of 2-D gels from seedlings with elevated CK levels and controls revealed significant differences in protein expression.
- High proportion of the differentially expressed proteins were related to oxidative stress and defense mechanisms including JA inducible proteins.
- Subsequent analyses revealed increased levels of hydrogen peroxide and JA in seedlings with elevated CKs together with significant changes in activity of several antioxidant enzymes.
- The data demonstrate, for the first time, a link between CKs and oxidative stress at the proteome level, implying cross-talk between CKs and JA in planta.

### References:

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

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