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# Gene expression in potato during cold exposure: Changes in carbohydrate and polyamine metabolisms

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ABSTRACT

Abiotic stress conditions, like water availability, temperature and salinity can have severe impacts on plant productivity. The present work focuses on the response to low temperature of three genotypes of potato (one *Solanum phureja* and two *S. tuberosum*). Measurements of the temperature inducing 50% of injured cells indicated that the *S. tuberosum* cultivar Desiree was not able to acclimate to cold, whereas the other cultivar acclimated to cold. *S. phureja* was more tolerant to cold on a constitutive level. Analyses of the transcriptome through cDNA microarray experiments coupled to real-time RT-PCR and of soluble carbohydrates, polyols and free polyamines as measured by HPLC, showed how these three genotypes of potato responded to chilling exposure. An array of genes involved in several metabolisms such as, e.g. amino acid, carbohydrate, energy, detoxification and photosynthesis were differentially expressed in cold exposed potato. On the metabolite level, carbohydrates accumulated in the three genotypes upon cold exposure and were constitutively more important in the *S. phureja* plant. Free polyamine metabolism was affected by cold as shown through an up-regulation of arginine decarboxylase, *S*-adenosylmethionine decarboxylase and spermidine synthase genes. Free polyamine accumulation was not pronounced upon cold exposure. Both molecular and biochemical results point to a differential response to cold exposure between the *S. phureja* and the *S. tuberosum* plants.

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## 1. Introduction

Cold, salt and drought stresses are environmental factors that have an important effect on growth, productivity and plant development [1]. They affect the water relations of plants at the cellular and whole plant levels causing specific as well as unspecific reactions, damages and adaptation reactions [1]. Interestingly, cold and drought stress share more common features with each other than with the salinity stress.

Low-temperature stress is one of the most critical environmental factors for plants and, like other abiotic stresses, it is responsible for economically important crop losses worldwide [1]. Plants can evolve and adapt to cold by increasing their tolerance through a process called cold acclimation. It can be achieved by an exposure to low non-freezing temperatures over a period of time. When plants are exposed to cold stress, many genes are induced; the products of these genes might either act directly against stress or indirectly by controlling the expression of other target genes [2].

Many studies have been performed to identify genes whose transcript levels are up-regulated in response to cold. Putative functions of these genes are dehydrins, kinases, genes involved in proline synthesis, pyruvate decarboxylase, to name a few. An exhaustive list is provided by Warren [3]. Fowler and Thomashow [4] also showed in an *Arabidopsis* transcriptome profiling study that multiple mechanisms are involved including differential expression of dehydrins and antifreeze proteins, chaperones and detoxification enzymes on one side and on the other side, changes in biosynthetic enzymes producing the previously described low molecular weight compounds. Moreover, regulatory proteins such as transcription factors, protein kinases and phospholipases are known to be differentially regulated upon cold stress.

Some of these genes are also induced by drought stress and to a lesser extent by salt stress. Generally, in cold-stressed plants, to avoid a deficiency in carbohydrates and thus avoid running out of cell energy, assimilates are redirected from supporting new growth to the synthesis of low molecular carbohydrates and soluble sugars, which can even increase the tolerance to further abiotic factors. These low molecular weight compounds are effective

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*Abbreviations:* ADC, arginine decarboxylase; PA, polyamine; Put, putrescine; SAMDC, *S*-adenosylmethionine decarboxylase; Spd, spermidine; Spm, spermine.

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osmoprotective substances [5]. Accumulation of carbohydrates (such as sucrose, glucose, fructose, raffinose or galactinol) upon drought, salt and cold stress in *Arabidopsis* has been shown in [6].

Photoinhibition is another case of metabolic imbalance [3]. Under stress conditions, photosynthesis does not limit growth, but instead, limited growth interferes with photosynthesis. Indeed, as a consequence for the decreased demand for assimilates, photosynthetic capacity is reduced.

Few reports clearly describe the involvement of endogenous polyamines (PA) in responses towards environmental stresses including cold stress. Shen et al. [7] describe a different effect on PAs when comparing cold-tolerant and cold-sensitive cucumber cultivars after chilling treatment. Free PAs, such as putrescine (Put), spermidine (Spd) and spermine (Spm) are thought to promote plant growth and development by, among others, the activation of nucleic acid and protein synthesis [8]. Recently, Rhee et al. [9] suggested that PAs could function as primordial stress molecules. Indeed, based on biochemical and genetic evidences, it was shown that free PAs, that are polycations, could function as scavengers for reactive oxygen species, acid tolerance factor and chemical chaperones. Moreover, they would be regulators for the expression of stress response genes [9].

The present work describes the response of potato exposed to chilling temperature. Microarray analyses were performed on three genotypes of potato, one *Solanum phureja* and two *S. tuberosum*. *S. phureja* is native to the Andes and performs well at high altitudes. In this paper, we investigate the effect of low-temperature on carbohydrate (saccharides and polyhydric alcohols) and PA metabolisms in potato leaves; discussion of the microarray results is restricted to functional proteins with emphasis on biosynthetic enzymes of the carbohydrate and PA metabolisms.

## 2. Materials and methods

### 2.1. Plant materials and stress treatments

Three potato genotypes were used to study the response to cold treatment: a diploid *S. phureja*, hereafter called CHS, a dihaploid *S. tuberosum*, hereafter called PS3, and a tetraploid *S. tuberosum* cv. Desiree. *In vitro* cultured plants were acclimated and grown *ex vitro* during 2 months in a mixture of soil:sand (3:1) before cold stress treatment.

Cold treatment of *ex vitro* potato plants was performed at 7 °C/2 °C (day/night) and control conditions at 21 °C/18 °C (day/night). Their morphological and biochemical reactions to cold are partly described in [10].

Cold-stressed and control plant leaves (1st and 2nd youngest leaves only) were sampled after 0, 1, 3 and 8 days.

Potato leaves were collected and stored at −80 °C until analysis.

### 2.2. Measurements of cold acclimation

The ability to cold acclimate was determined as described by Renaut et al. [11], according to Lim et al. [12]. It was determined after a treatment period of 0 and 21 days at 4 °C by measuring changes in electrical conductivity of the leaves and estimated by the $LT_{50}$ corresponding to the temperature inducing 50% of injured cells. Eight leaves were used for each assay. Samples were gradually cooled in a temperature-controlled bath (Julabo Labortechnik, F12-MP). Tubes were removed at a selected temperature and allowed to reach room temperature; then 40 mL of double distilled water were added. Tubes were shaken for 2 h at room temperature and conductivity was measured. Tubes containing leaves and water were autoclaved (0.12 MPa, 120 °C, 20 min) and, after cooling, conductivity was again measured. The percentage of ion leakage and injury was determined according to [13]. From plotting the percentage of injury as a function of treatment temperature, the temperature at which 50% of the cells were injured was obtained.

### 2.3. cDNA microarrays

For microarray analyses, sample time points 1 and 3 days after the onset of cold stress were selected for cold-treated and control plants.

RNA was extracted by the RNeasy plant mini kit (Qiagen, Leusden, The Netherlands) including DNase treatment, according to the manufacturer's instructions.

Purity of the total RNA extracted was determined as the 260 nm/280 nm ratio and the integrity was checked by electrophoresis in 1% agarose gel.

Aminoallyl labelling of RNA for microarrays was performed with 20 μg of total RNA according to TIGR's protocol http://pga.tigr.org/sop/M004_1a.pdf. To remove the unincorporated aa-dUTP and free amines, the Microcon YM-30 (Millipore, Saint Quentin en Yvelines, France) cleanup method was chosen. To remove uncoupled dye Qiagen MinElute PCR Purification kit (Qiagen, Leusden, The Netherlands) was used and the elution was performed twice with 10 μl each of EB buffer.

Probe hybridization was performed according to TIGR's protocol http://pga.tigr.org/sop/M005_1a.pdf with some modifications. The slides were dried by centrifugation at 500 rpm during 5 min instead of blow-drying the slides with compressed air. The labelled probes were re-suspended in 41.5 μl of 1× hybridization buffer and to block non-specific hybridization, 1.3 μl of salmon sperm DNA (10 mg/mL), and 1.7 μl of Poly(A)-DNA (20 μg/μl) were used.

The experimental set-up was such that differentially expressed genes according to genotype and to cold exposure at 1 and 3 days could be identified. Cold samples (labelled with Cy5) were hybridized versus control samples (labelled with Cy3) for each time and each genotype. Two independent experiments were performed and for each experiment two slides, for each genotype and each cold time, were performed using one leaf of two different plants. TIGR 10K potato microarrays http://www.tigr.org/tdb/potato/microarray_desc.shtml were used in these experiences; these slides contain 15,264 cDNAs repeated twice. Hereafter, genes will be described by their clone name as given by TIGR (STM code).

Microarray slides were scanned with the GenePix® 4000B microarray scanner (Molecular Devices Corporation, CA, USA). The slides were scanned with two laser channels (532 and 635 nm) at different settings for the photo multiplier tube (PMT Gain). The two PMT Gains were adjusted automatically by Genepix® Pro6.0 software to have a global intensity ratio close to 1.

To analyze gpr files generated by Genepix® Pro6.0 software, data were imported into Acuity 4.0 software (Molecular Devices Corporation, CA, USA). Data were normalised with the lowess normalisation using Acuity software (M&A plots were performed to check the quality of the data).

### 2.4. Primer design for RT-PCR

Primers were designed with Primer Express® 2.0 (Applied Biosystems, Foster City, USA) such as to obtain a 15–25 bp primer length with GC content between 40% and 60% avoiding hairpins and complementarity between primers. Primers chosen have an annealing temperature between 59 and 61 °C.

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