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# Cold-modulated expression of genes encoding for key enzymes of the sugar metabolism in spring and autumn cvs. of *Beta vulgaris* L.

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## Abstract

An integrated approach based on the use of bioinformatics and gene expression analysis tools was carried out to evaluate the organ-specific transcription modulation of nine genes relevant to sugar metabolism of *Beta vulgaris* L. plantlets of the autumn cv. Franca and spring cv. Bianca, in response to low-temperature (LT) treatments. Different growth cycles imply different plant capability to adapt to the environment that includes variations in gene expression of key metabolic enzymes. The transcriptional response was evaluated by quantitative PCR analysis before, during and after the LT treatments. The results were correlated with the LT-induced electrolyte leakage measure and the carbohydrate content. Stress-induced transcript level alterations were detected in the two cultivars, suggesting a modulation of sucrose synthesis and carbohydrate partitioning. Cold stress induced deep changes in the autumn cultivar, especially in fructose-1,6-biphosphatase gene expression, irrespective of temperature or exposure time. These differential features of expression profiles constitute first clues on the molecular basis of the differential LT response of sugarbeet autumn and spring cultivars.

**Keywords:** *Beta vulgaris*; cold stress; real-time PCR; sucrose metabolism; sugarbeet

## Introduction

Cold stress is a significant cause of crop losses in European and North American agriculture, conditioning crop quality and production. The early sowing of sugarbeet (*Beta vulgaris* L.), to improve root production and escape drought periods at maturity, and the autumn sowing, a practice adopted in Southern Europe, expose young plantlets to freezing temperatures (below 0°C) and to the risk of severe crop and quality losses. Nevertheless, the response to low temperature (LT) changes according to the genotype, suggesting the existence of genetic variability among the different varieties.

Many different sugarbeet varieties are available for cultivation in different environments, and with specific adaptation to different sowing times, as the two commercial varieties employed in this study: cv. Bianca, selected for spring sowing and characterized by high germinability and tolerance to cercospora, and cv. Franca, selected for autumn sowing and tolerant to bolting.

The productivity in sugarbeet is a complex trait and depends on sucrose content (SC) and yield (SY). The quantitative trait-corrected SY, comprehensive of both SC and SY, is the result of the activity of different enzymes: sucrose synthases (SBSS1 and SBSS2), sucrose phosphate synthases (SPS1 and SPS2), fructose bisphosphate aldolase (FBPald), fructose bisphosphatase (FBPase) and choline monooxygenase (CMO) (Schneider *et al.*, 2002).

With the aim to understand the adaptation of sugarbeet crop to the environment, the response to cold stress of

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two different cultivars of *B. vulgaris*, selected for early and late sowing respectively, was investigated employing molecular, physiological and metabolic approaches, focusing specifically on metabolic pathways involved in the control of sucrose biosynthesis/degradation and distribution in the different tissues of the plant.

## Materials and methods

Seedlings of diploid sugarbeet (spring cv. Bianca and autumn cv. Franca) were hydroponically grown; the LT treatments, chosen accordingly to the electrolyte leakage (EL) test results, and the samplings are reported in Table 1. Each experiment was replicated twice. The specific intron-spanning primers and probes (TaqMan; 'Assay by Design') were obtained interrogating the institute for genomic resources *B. vulgaris* Gene Index database, by the selection of tentative consensus functionally annotated with high significance by BLAST X (similarity above 90%; Supplementary Table S1, available online only at http://journals.cambridge.org).

Changes in the relative quantification of mRNA levels of nine target genes were analyzed by a two-step real-time PCR analysis (ABI PRISM 7000; Applied Biosystems). For their relative quantification, the geometric mean of the most suitable reference genes (rRNA18S and tubulin, data not shown) was used, in order to compensate for small expression fluctuations, according to Nicot *et al.* (2005). The fold-change in gene expression was calculated by relative quantification method of comparative Ct($2^{-\Delta\Delta Ct}$), according to Livak and Schmittgen (2001) and checked for statistical significance by *P* value and standard deviation of ΔΔCt, according to Yuan *et al.* (2006).

Glucose, fructose and SC in leaves and roots of control and treated plantlets, pot-grown in controlled conditions for 2 months, were determined by HPLC-evaporative light scattering detector (ELSD).

## Results and discussion

The response to cold stress tests showed that LT injury – measured as EL of excised segments of leaf – increased in the range from 0 to −8°C for both cvs., and that −2°C was a temperature at which about 25–40% of tissue damage could be observed. Besides, cv. Franca showed a lower electrolyte release in the solution in the range between −2 and −4°C (Supplementary Fig. S1, available online only at http://journals.cambridge.org), making the two cvs. a good model to study LT response.

Expression level of nine target genes (Supplementary Table S1, available online only at http://journals.cambridge.org) in untreated tissues (leaves and roots)

**Table 1.** Experimental schedule[1]

| | | | Temperature and exposition times | | | | |
|---|---|---|---|---|---|---|---|
| | | | Sampling times | | | | |
| Experiment | Growth period | Acclimation | 1 ↓ | 2 ↓ | 3 ↓ | 4 ↓ | |
| I | 23°C/17°C 24 d | No | 23°C/17°C, 3 h | 23°C/17°C, 5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Calibrator |
| II | 23°C/17°C 24 d | 6°C/8 h, three nights | 23°C/17°C, 3 h | 23°C/17°C, 5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Calibrator |
| III | 23°C/17°C 24 d | No | 0°C/3 h | 0°C/5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Target |
| IV | 23°C/17°C 24 d | 6°C/8 h, three nights | 0°C/3 h | 0°C/5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Target |
| V | 23°C/17°C 24 d | No | −2°C/3 h | −2°C/5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Target |
| VI | 23°C/17°C 24 d | 6°C/8 h, three nights | −2°C/3 h | −2°C/5 h | 23°C/17°C, 24 h | 23°C/17°C, 7 d | Target |

[1]Plantlets of sugar beet (spring cv. Bianca and autumn cv. Franca) were hydroponically grown for 4 weeks in controlled conditions. The LT treatments (0 and −2°C) were applied for 5 h and during each experiment (named from I to VI), the samples (leaves and roots) were collected four times (arrows): during (at 3 and 5 h) and after LT treatment (24 h and 7 d). The experiments II, IV and VI included a short acclimation period (6°C/8 h for three nights).

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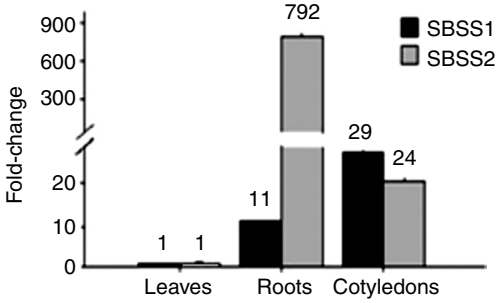
(a)




(b)

| | B Leaves | B Roots | F Leaves | F Roots |
|---|---|---|---|---|
| SBSS1 | 1 | 11 | 1 | 14 |
| SBSS2 | 1 | 792 | 1 | 104 |
| SPS1 | 1 | 54 | 1 | 4 |
| SPS2 | 1 | 28 | 1 | 6 |
| FBPase | 7 | 1 | 1 | 22 |
| FBPald | 1 | 2 | 1 | 2 |
| PDHkin | 2 | 1 | 5 | 1 |
| CMO | 9 | 1 | 1 | 27 |
| DREB2A | 1 | 500 | 1 | 200 |

**Fig. 1.** (a) Transcript levels of the two sucrose synthase isoforms (SBSS1 and SBSS2) in leaves, roots and cotyledons of control sugarbeet plants. Expression was normalized on the geometric mean of rRNA 18S and tubulin, using leaf as calibrator. (b) Fold-change of all the target genes analyzed in Bianca (B) and Franca (F) in leaves and roots of untreated plantlets; in each case, the transcript level of the organ in which the expression was lower has been used as calibrator.

of both cvs. are reported in Fig. 1(c). For the two sucrose synthase genes, low transcriptional levels were observed in the leaves of both cvs., while in roots, especially SBSS2 was strongly transcribed, as also reported by Hesse and Willmitzer (1996); Fig. 1(a, b).

The induction of the two osmotic stress marker genes (CMO and DREB2A) proved the transcriptional response of sugarbeet organs to LT treatments used in the experimental system (Supplementary Figs. S2 and S3, available online only at http://journals.cambridge.org): CMO transcription showed a strong increase in roots of cv. Bianca, in the late phase, after the exposition to −2°C, suggesting an accumulation of the osmoprotector glycinebetaine, in order to maintain the osmotic potential inside the root cells, as observed also by Pestsova *et al.* (2008).

The time course of modulation of the genes coding for enzymes of carbohydrate metabolism upon exposure to LT revealed a different behaviour in the two sugarbeet cultivars in terms of response onset and gene induction/repression. The differences were detectable mainly in the early phase, after 3–5 h from stress application, when the LT stress was applied as a shock (Supplementary Figs. S2 and S3, available online only at http://journals.cambridge.org). Especially, in the leaves of Bianca, SBSS1 and SPS1, transcription responded promptly, though transiently, to the exposure to LT, suggesting their implication in a early response to cold stress; in Franca cv., SPS1 was mainly modulated after an acclimation at 6°C followed by exposure to acute temperature stress, differently from Bianca. The most important step of sucrose synthesis/degradation is regulated by fructose 1,6 biphosphatase controlling the resynthesis of glucose from sucrose (Nielsen *et al.*, 2004) and fructose biphosphate aldolase catalyzing the conversion of dihydroxyaceton-phosphate and glyceraldehyde 3-phosphate in fructose 1,6-bisphosphate. Their expression profiles did not change significantly in roots and leaves of cv. Bianca, while in cv. Franca, it was found that the cold stress, irrespective of temperature or exposure time, induced deep and fixed changes, mainly in fructose 1,6 bisphosphatase (Supplementary Fig. S4, available online only at http://journals.cambridge.org) expression, suggesting a key role of the enzyme in sucrose metabolism regulation and outlining a cultivar-dependent response specificity.

The hardening response, triggered by gradual exposition to low but not injuring temperatures, has never been observed in sugarbeet. We registered however, a differential behaviour in the two cultivars examined upon night exposure to 6°C, followed by a further exposure to 0 and −2°C.

Here, we detected stress-induced alterations in gene expression, suggesting an induction at the transcriptional level of sucrose synthesis and a reduction of carbohydrate partitioning during and after cold stress. These differential features of LT-response expression profiles in cultivars selected for different climates and susceptibility to cold deserve further analysis, and were previously unknown. The above-described changes are, to some extent, similar to those described in *Arabidopsis thaliana* leaves undergoing LT stress (Guy *et al.*, 2008), suggesting a general conservation of the mechanism in dycotyledons. Actually, consistent expression variations in the majority of the analyzed genes have been reported mainly in cv. Franca, suggesting these gene expression levels a possible factor contributing to its suitability for autumn sowing.

The different expression profiles detected for both cvs. reflected a different behaviour, evident also in the different tolerance of leaf and root to LT, suggesting a superior performance of cv. Franca; moreover, this autumnal cultivar showed, in all conditions analyzed, a decreased expression of FBPase, especially in roots, suggesting that triose-phosphate pool was directed towards the glycolytic pathway, i.e. energy production, rather than

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towards glucose biosynthesis and consequential sucrose accumulation, to better cope with stress conditions.

HPLC-ELSD analysis showed that glucose and fructose contents of leaves and rootlets did not change after LT treatments in cv. Bianca compared with the control plants (Supplementary Fig. S5, available online only at http://journals.cambridge.org), suggesting that early cold exposure of plantlets does not affect sucrose production in sugarbeet.

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