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Research article

Transcriptome analysis in pea allows to distinguish chilling and acclimation mechanisms

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ABSTRACT

In order to distinguish chilling and freezing tolerance mechanisms in pea, responses to cold exposure were compared between the freezing tolerant line Champagne and the sensitive line Terese. Global gene expression was considered in the two lines and associated with morphological, histological and biochemical approaches. The chilling tolerance in both lines was related to responses of the *CBF*, *COR* and *LEA* genes belonging to the CBF regulon, with greater earliness of expression in the Champagne genotype. The freezing tolerance, only observed in Champagne, was associated with acclimation processes such as cellular osmotic stabilization, photosynthesis modifications, antioxidants production, modifications in hormone metabolism, cell wall composition and dynamics.

1. Introduction

Cold is an abiotic stress affecting plants and has triggering consequences on their geographical distribution. Low temperatures results to a decrease yield and also affects the quality of harvests, with substantial economic losses at world level. For this reason, many studies are being conducted to understand the effects of exposure to cold and the mechanisms that allow plants to tolerate cold and freezing [1].

Cold stress damages plants through temperature and exposure time [2]. Low non-freezing temperatures lead to lower photosynthesis activity, increased membrane rigidity that can lead to a loss of electrolytes, and changes in enzymes functioning. Freezing temperatures can bring about the formation of intracellular ice, tears and fusions of cellular membranes with the sudden occurrence of frost, or cellular dehydration before membrane injury when the temperature decrease is progressive [1].

Winter tolerance includes inducible stress tolerance mediated by exposure to reduced temperatures known as chilling tolerance and cold acclimation. Chilling tolerance represents the ability of a plant to respond to low but positive temperatures in the range 0–15 °C. Cold acclimation represents the capacity of plants to tolerate negative temperatures and occurs when plants had been previously exposed to low, but still positive temperatures. This acclimation process involves many more metabolic changes in comparison to chilling tolerance [1,3]. Changes of primary metabolisms and/or biosynthesis of new proteins, cellular osmotic stabilization, changes in lipid membrane composition, increase in antioxidants, were described during cold acclimation in numerous dicots such as alfalfa [4], *Arabidopsis thaliana* [5], and poplar [6] and in monocots such as rye [7] and wheat [8]. Osmolyte protection was studied for decades in correlation with plant cold acclimation [9] and consists in the accumulation of polypeptides with osmoprotective properties, such as COR15a [10], and synthesis of low molecular-weight osmoprotectants such as proline or raffinose [11,12].

Winter tolerance in pea (*Pisum sativum* L.) was associated also with allelic variation at the *Hr* (*High response*) locus, which plays an

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Abbreviations: CBF, C-repeat binding factor; COR, cold-regulated genes; LEA, late embryogenesis abundant proteins.

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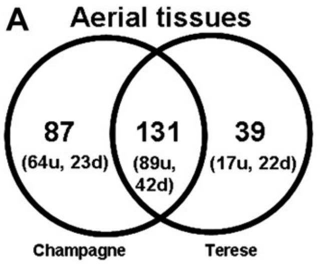

Fig. 1. Venn diagram representing the number of commonly and differentially expressed genes between pea genotypes during LT-treatment. A: common and specific response in aerial tissues; B: common and specific response in roots; u: up-regulated genes; d: down-regulated genes. Genes up- or down-regulated for at least one of the 4 points in the time course of the cold stress experiments were counted.

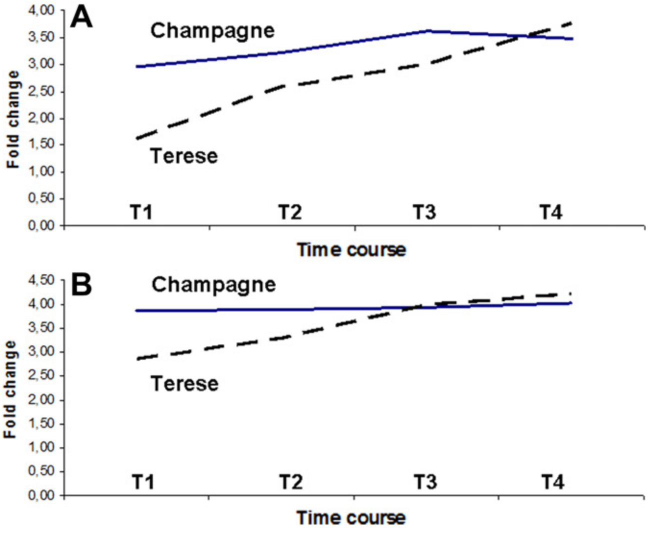

Fig. 3. Functional categories presenting differences in time course expression of genes involved in pea chilling responses. A: Course time response of genes involved cell cycle and DNA processing, cellular transport, cellular communication and signal transduction shows an early expression in Champagne and only late expression in Terese. B: Course time response of genes involved in cell rescue and interaction with the environment shows a constant expression in Champagne and only late expression in Terese. Fold change average of gene expression was calculated for both groups of functional categories.

important role in the response of floral initiation [13]. Pea is also known to present an acclimation responses during vegetative phasis [14]. Therefore in the present work, the aim was to obtain better global understanding of these different mechanisms by performing a transcriptomic study associated with morphological, histological, and biochemical measurements in different tissues of pea subjected to cold stress. Indeed, the use of two pea genotypes, one freezing tolerant (Champagne) and the other freezing sensitive (Terese), represented an efficient tool for discriminating chilling from freezing tolerance responses.

2. Results and discussion

Profiles of gene expression together with morphological, histological and biochemical observations performed in Champagne and Terese, suggested that their chilling and acclimation responses are qualitatively and/or quantitatively different.

Among the 4946 genes represented on the microarrays, the expression of 257 genes was modified during LT-treatment in aerial tissues (Fig. 1A). Functional categories for these genes were defined in Supplemental data file 1.

In the Champagne genotype, there were twice as many transcriptome modifications as those of Terese in the aerial tissues (87 genes differentially expressed in Champagne compared to 39 in Terese (Fig. 1)A and 6 times more in the roots (35 genes differentially expressed in Champagne compared to 6 in Terese (Fig. 1B).

Gene-specific quantitative qRT-PCR was used for the cross-validation of microarray performance. We selected 8 genes reflecting up- and down-regulated genes in both pea lines or specifically regulated in Champagne or Terese during LT-treatment. The high concordance between microarray and qRT-PCR data (coefficient of correlation (r) between 0.9816 and 0.9960) indicated that microarray platform was able to accurately predict the change of gene expression level between subsets of interest (Supplemental data file 2).

2.1. *Common responses*

134 genes that were found similarly involved in LT response in aerial and root tissues in both pea genotypes: these genes are involved in amino acid metabolism, carbohydrate or lipid metabolisms, secondary metabolism, signalling and signal transduction, cellular defence, cold stress responses and cell wall biogenesis (Supplemental data files 3 and 1).

The CBF-regulon, together with carbohydrate and amino acid metabolisms, are among the most reported functional responses to

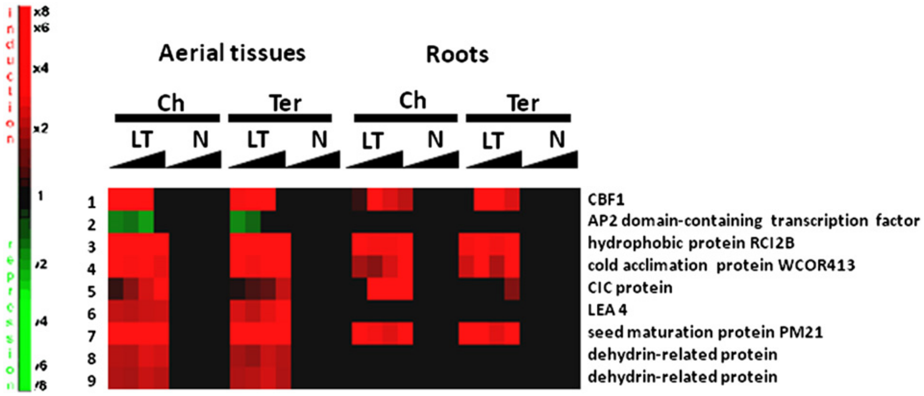

Fig. 2. Cluster representing modifications in the expression of genes belonging to the COR-regulon in pea during N- and LT-treatment. Cluster was constructed on the basis of log2ratio of gene expression in each condition *vs.* corresponding T0 values. Triangles represent time-course modifications in gene expression (from T1 to T4). Ch: Champagne; Ter: Terese; LT: chilled experiment; N: unchilled experiment.