Low Temperature Conditioning of Garlic "Seed" Cloves Induces Differential Response in Sprouts Proteome

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Introduction

Low-temperature conditioning of garlic "seed" cloves at 5 °C for five weeks substitutes the initial climatic requirements of the crop and accelerates the development of the crop cycle. Also, reduces growth and plant weight as well as the crop yields and increases the synthesis of phenolic compounds and anthocyanins in the bulbs at harvest time. Plant acclimation to low-temperature is associated with deep changes in proteome composition. Since proteins are directly involved in plant response to environmental conditions, proteomics studies can significantly contribute to unravel the possible relationships between protein abundance and low-temperature acclimation.[1]

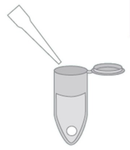
Objective

The aim of this work was to study the changes in the protein profiles of garlic "seed" cloves subjected to conditioning at low-temperature using proteomics approach.

Materials & Methods

1 Conditioning

'Coreano' garlic harvested in 2011-12 season was used. The 'Seed' cloves were stored at room temperature (RT) and 5°C during 5 weeks. Garlic sprouts were taken and freeze at -80°C.

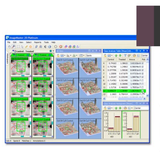


2 Protein extraction

Powdering under N_2, acetone washing, extraction with lysis buffer (8M Urea, 2% CHAPS, 20 mM DTT, 2mM PMSF, 0.002% bromophenol blue) and clean up with RC reagents package (Bio-Rad).

3 2 DE

Separation by IEF using 24 cm, pH 4-7 strips and by MW using 13% acrylamide gels. 2-DE gels were stained with Coomassie blue R-350. Three biological replicate gels per condition were run.

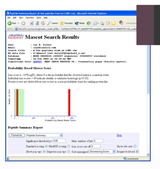


4 Image analysis

Acquired using a Pharos FX Imager (Bio-Rad). Analysis was performed with Melanie v7.0 those spots with statistically significant changes (t-test with a $P<0.01$) were considered to be differential protein spots.

5 LC ESI MS/MS

Selected protein spots were excised from the gels and distained, reduced and digested with trypsin. Peptides were separated and analyzed by LC-ESI-MS/MS (ACQUITY UPLC-SYNAPT HDMS, Waters)



6 Identification

Conducted against the *Viridiplantae* subset of the NCBInr protein database and against the *A. sativum* subset of the NCBInr EST database using PKL files and the Mascot search engine v2.3 ($P<0.05$).

Results & Discussions

Figure 1 illustrates the sixty-two protein spots that showed statistically significant changes in abundance between treatments and were analyzed by LC-ESI-MS/MS. 81% of the spots were identified by database search analysis using the Mascot search engine.

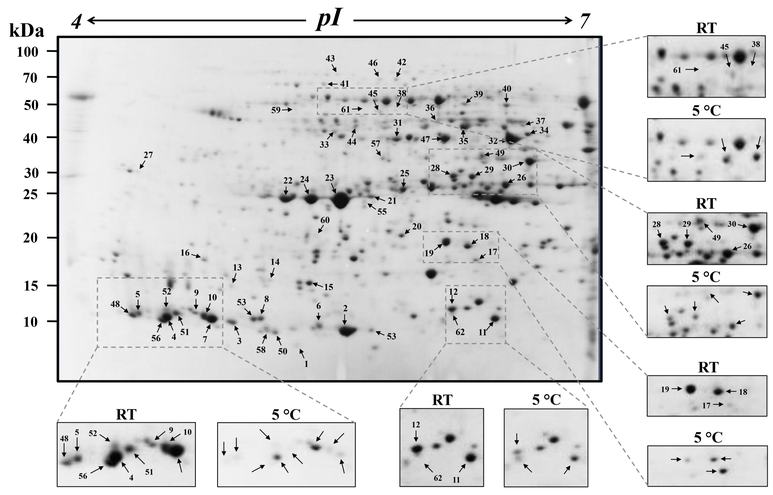


Figure 1. Representative 2-DE pattern of garlic sprouts proteins from cloves stored at room temperature (RT) or low temperature conditioning (5°C).

According to Gene Ontology these proteins were grouped into nine different categories in function to the biological processes in which they are involved; cellular response to stress, carbohydrate binding, regulation of transcription, transport of macromolecules, protein folding, photosynthesis, carbohydrate metabolism, nucleotide metabolism, and miscellaneous (**Figure 2**).

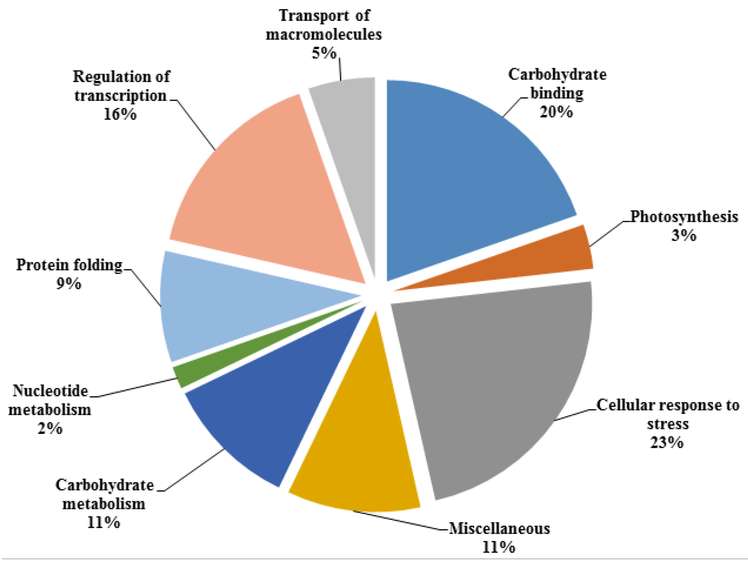


Figure 2. Classification of the identified proteins, the pie chart shows the distribution of the low temperature conditioning responsive proteins into their biological process in percentage according to Gene Ontology

The results indicated significantly increased protein spots including RNA-binding protein, chaperone and S-adenosylmethionine synthetase. Significantly decreased protein in garlic sprouts spots subjected to cold conditioning, which include mannose-specific lectin, macrophage migration inhibitory factor, annexin D2-like and glutathione S-transferase.

A hypothetical model is proposed to illustrate cellular events potentially associated with the effects of low-temperature conditioning (**Figure 3**).

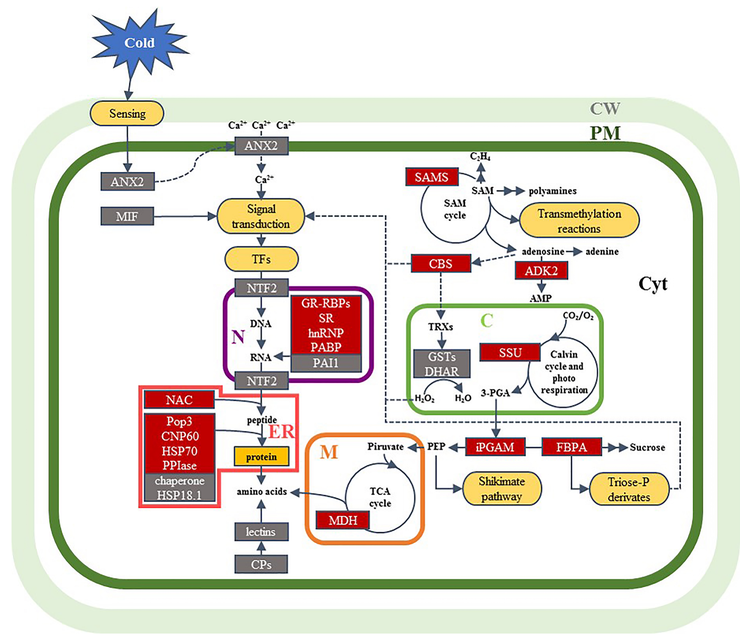


Figure 3. A model summarizing the effects of cold conditioning into a garlic sprout cell derived from the changes observed at proteome level.

The data presented in this research indicate that the low-temperature conditioning of "seed" garlic cloves during 5 weeks at 5 °C affected different metabolic pathways and physiological processes. These processes can work cooperatively to establish a new cellular homeostasis that might be related with the physiological and biochemical changes observed in previous studies.[2,3]

Perspectives

This is the first work that reports the changes in the protein profiles of garlic "seed" cloves to low-temperature conditioning.

The identification of cold-responsive proteins in garlic provides not only new insights into cold conditioning responses but also a good starting point for further dissection of their functions during the development of the crop using genetic and other approaches.

References

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2. Dufoo-Hurtado, M. D., *et al.* 2013. J. Agric. Food Chem. 61. 10439–10446.
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Acknowledgments

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