Identification of Proteins that Enhance Frost Tolerance Utilizing a 2D-DIGE Approach

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Introduction

Un-seasonal frosts are a major restraint to crop production in Canada. Crops such as canola, are very frost sensitive. However, when exposed to low temperature (LT), they can increase their frost tolerance. During exposure to low non-freezing temperatures, genes are up-regulated or down-regulated to allow for changes in cell structure, cytoplasm and metabolite composition and hormone balance. The role of cold-induced proteins is to protect the cells from freezing-induced desiccation due to the formation of ice within their tissues. These proteins fall into three major classes: transcription factors, cryoprotective proteins and cell detoxifiers. Transcription factors induce and regulate downstream low temperature associated genes and gene families. For example, a C-repeat Binding Factor (CBF), binds to a C-repeat region present in the promoter of COld Responsive (COR) genes. Dehydrins, a family of cryoprotective proteins (LEA D11 proteins) form complexes with proteins or cellular structures to prevent denaturation during freezing. Metabolism under low temperatures results in a drastic increase in free radical molecules causing nucleic acid, protein and membrane disruption. SuperOxide Dismutase (SOD) metabolizes superoxide into hydrogen peroxide, a less toxic molecule which the cell can deal with.

The proteome of the plant differs based on the type of LT exposure as well as its frost tolerance. We compared the proteomes of freezing-sensitive (no LT exposure), freezing-tolerant (23days at below 10ºC, non-natural cold-shocked (24h at 2ºC; directly transferred) and near-natural cold-stressed (24h at 2 ºC; gradually cooled) canola.

Using the Ettan™ DIGE and the Ettan MDLC systems, we have been able to quantify changes in protein expression specific to each type of LT exposure, as well as those common to all, which have a high probability of being frost-associated proteins.

Methods

Brassica napus seedlings were exposed to four different LT treatments as discussed above. The leaves were ground and then solubilized in the following buffer: 7M Urea, 2M Thiourea, 4% CHAPS, 1% Ampholytes, 18mM Tris, 14mM Trizma, 1 Complete mini Protease Inhibitor Tablet (Roche), 530U RNase, 2%Triton X-100, 20mM DTT. Samples were cleaned up using the PlusOne™ Clean-Up Kit and labeled with CyDyes using the standard DIGE protocol, which utilizes a pooled internal standard.

The samples were then randomized on 12 Immobiline™ DryStrips IPG strips (24cm, 3-10NL) and run on an IPGphor II™. The second dimension was run on the Ettan DALT*twelve* system. Differential protein analysis was performed using DeCyder™ 2D Differential Analysis Software 6.0. Proteins were selected for identification based on the following three parameters: Average Ratio >1.5 and <1.5, T-test p-value <0.05, 1-ANOVA <0.05. Protein identifications were obtained using the Ettan MDLC and the Finnigan™ LTQ equipped with an electrospray ion source.

Results

The results of this analysis showed changes in each sample that spanned both pI and molecular weight ranges. No obvious localization of changes, or that of changes of similar magnitudes, were present. However, a large number were detected that exhibited 1-way ANOVA and T-test *p*-values that were substantially lower than the chosen parameter of 0.05, giving confidence that these were actual changes. In addition, a high percentage of the detected changes represented proteins that were seen in two or more of the samples.

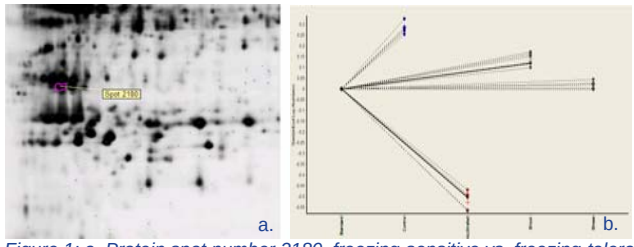

Figure 1: a. Protein spot number 2180, freezing-sensitive vs. freezing-tolerant b. Graph showing log relative abundance of spot 2180 on each of the 12 gels grouped according to sample treatment

	Tolerant vs. Sensitive	Tolerant vs. Stressed	Tolerant vs. Shocked
Total changes	372	175	80
Total shared changes	91	119	39
Shared with Acclimated	-	86	44
Shared with Stress	-	-	64

Table 1: Summation of changes in protein expression between the samples and the control that met statistical requirements. "Shared" proteins exhibited changes in more than one sample.

Conclusion

- Number of changes in protein expression correspond to length of low-temperature exposure, i.e. longer exposure leads to increase in changes
- While freezing-sensitive showed most changes in expression, Stress exhibited the most changes shared between the different groups
- Statistics from DeCyder software provided confidence that changes were real
- Further analysis will help to identify specific proteins, or groups of proteins, that facilitate cold acclimation

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