In the quest for a cold tolerant variety

gene expression profile analysis of cold stressed oat and rice

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AKADEMISK AVHANDLING

för filosofie doktorsexamen vi Göteborgs Universitet, kommer att försvaras offentligt i sal 'Tor Bjurström', Medicinaregatan 3, fredagen den 5 december, 2008, klockan 13.00

Faculty opponent: Professor Joachim Selbig, Max Planck Institute of Molecular Plant Physiology and University of Potsdam, Potsdam, Germany

Avhandlingen baseras på följande arbeten:

- I. Bräutigam, M., **Lindlöf, A.**, Zakhrabetkova, S., Gharti-Chhetri, G., Olsson, B. and Olsson, O. (2005) Generation and analysis of 9792 EST sequences from cold acclimated oat, Avena sativa. *BMC Plant Biology* 5:18.
- II. Lindlöf, A., Bräutigam, M., Chawade, A., Olsson, B. and Olsson, O. (2007) Identification of Cold-Induced Genes in Cereal Crops and Arabidopsis through Comparative Analysis of Multiple EST sets. In: Hochreiter, S. and Wagner, R. (eds.), *Bioinformatics Research and Development – First International Conference, BIRD '07*, LNBI 4414: 48-65. Springer-Verlag.
- III. Lindlöf A., Bräutigam M., Chawade A., Olsson O. and Olsson B. (2008) Evaluation of combining several statistical methods with a flexible cut-off for identifying differentially expressed genes in pairwise comparison of EST sets. *Biology and Bioinformatics Insights* 2: 215-237.
- IV. Bräutigam, M., Lindlöf, A., Chawade, A., Gharti-Chhetri, G., Olsson, B. and Olsson, O. (2008) Transcriptional profiling of cold stress response in rice and comparative analysis to *Arabidopsis thaliana* (manuscript).
- V. Lindlöf A., Bräutigam, M., Chawade, A., Gharti-Chhetri, G., Olsson, B. and Olsson, O. (2008) *In silico* analysis of promoter regions from cold-induced *CBFs* in rice (*Oryza sativa* L.) and *Arabidopsis thaliana* reveals the importance of combinatorial control (manuscript).

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Abstract

Cold acclimation is a process which increases the freezing tolerance of an organism, after exposure to low, non-freezing temperatures. The acclimation ensures that cold tolerant species can endure harsh winter conditions, by preparing them to sub-zero temperatures. Cold-sensitive plants such as oat and rice have limited abilities to cold acclimate and are therefore easily damaged during winter time.

The development of more tolerant varieties by using biotechnological methods is desirable, since the yields are expected to improve due to a prolonged vegetation period. However, in order to apply such methods, more knowledge about the underlying mechanisms regulating the cold tolerance and acclimation is required. One step in this direction is to analyze gene expression data generated from cold stressed oat (*Part I*) and rice plants (*Part I*).

The focus of this thesis is, consequently, analysis of expression profiling data, which was generated using the EST sequencing and cDNA microarray technologies. The results show that both oat and rice are cold responsive, with many of the previously identified cold regulated genes having a counterpart in these species. In rice, however, the response is less dynamic than in the model organism *Arabidopsis thaliana* and this may explain its inability to fully cold acclimate.

Additionally, the work in this thesis focuses on evaluating if small-scale EST sets can be used for 'digital-Northern', in order to identify genes that are involved in the regulation of the cold stress response. The results show that small-scaled EST sets are not optimal for such an analysis, since the method detected only a portion of cold responsive genes represented in the sets. This has to due with the inherent properties of EST data and limitations in the analysis steps of the sequences.

The work also concerns the identification of *cis*-elements coupled to transcription factors prominent in the regulation of the response. Since cold acclimation is a quantitative trait the response and regulation of cold stress is under combinatorial control of several transcription factors and the results show that this should be taken into account when identifying binding sites.

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