Isolation of candidate genes involved in cold temperature response in *Festuca pratensis* Huds., using suppression subtractive hybridisation and microarray approaches

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Introduction The objective of this work was to isolate candidate genes which are differentially expressed following cold-acclimation and develop SNPs to test for associations between candidate genes and frost tolerance. The ability to develop sufficient levels of tolerance against freezing temperatures through cold-acclimation (hardening) is crucial for survival of grasses and winter cereals in temperate climate. Meadow fescue (*Festauca pratensis* Huds.) is one of the most important forage grass species in Northern Europe. The preference of *Festuca* instead of *Lolium* in Norway is due to its superior combination of winter hardiness and forage quality.

Materials and methods Cold-acclimated and non-treated *Festuca* crown tissue of plants with low and high frost tolerance were used. RNA was isolated and cDNA libraries were prepared using suppression subtractive hybridisation (Clontech PCR-Select cDNA subtractive kit). 559 ESTs from the cDNA libraries were sequenced and spotted on a microarray. Expression profiling using mRNA pools isolated from individual plants of meadow fescue populations selected for high and low frost tolerance was conducted.

Results The genome of *Festuca pratensis* is not sequenced and in order to get useful information from our cDNA libraries we performed Blast searches of the 559 ESTs against the NCBI database. Through these searches we identified 242 homologous sequences in other species, 131 of our ESTs were homologous to putative proteins in other species, 78 to sequenced clones with no predicted protein, 81 with no significant similarities in the database and 27 homologous to unknown proteins in other species. We found homologues involved in cold stress but also other stress related genes encoding enzymes involved in several metabolic pathways, ribosomal proteins, histones, ubiquitin, elongation factors and others. The GeneSpring expression analysis software was used to analyze the expression profiling data. In the high frost tolerant hardened (Hi-H)/high frost tolerant non-hardened (Hi-NH) hybridisation 111 genes were found to be upregulated and 36 down-regulated more than 2-fold (Figure 1). Candidate genes involved in frost tolerance were found among these genes. Comparison of Hi-H and low frost tolerance hardened (Lo-H) genotypes showed 4 genes to be up-regulated and 3 down-regulated more than 2-fold (Opseth 2004).

Conclusions The results of the present study demonstrate that SSH in combination with a cDNA microarray is a successful approach for candidate gene isolation. All the candidate genes that were among the SSH-cDNA libraries were found to be differentially expressed upon cold-acclimation of high frost tolerant *Festuca* genotypes. The results will improve our understanding of the genetic regulation of cold-acclimation and frost-tolerance in forage grasses and cereals, and provide functional allele-specific markers that can be implemented in molecular breeding strategies.
(http://www.grasp-euv.dk/default.asp)

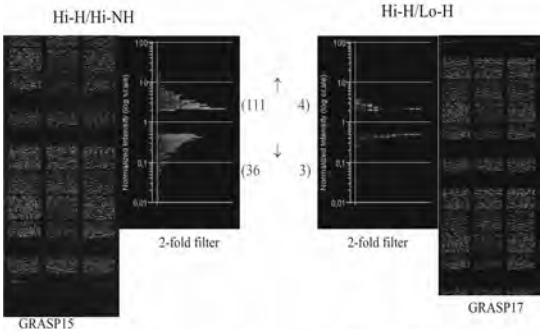

Figure 1 Composite images of two hybridisations and a statistical plot showing a 2-fold filtering of the expression data

Reference

Opseth, L (2004) Isolation of genes involved in cold-tolerance in *Festuca pratensis* Huds. by suppression subtractive hybridization and microarray. Cand. Scient. Thesis, Agricultural University of Norway