Norway spruce deploys tissue-specific responses during acclimation to cold

Alexander Vergara[1], Julia Haas[1], Tuuli Aro[2], Paulina Stachula[1], Nathaniel Street[3], and Vaughan Hurry[2]

[1]Umeå Plant Science Centre
[2]Sveriges lantbruksuniversitet
[3]Umeå University

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Abstract

Cold acclimation in plants is a complex phenomenon involving numerous stress-responsive transcriptional and metabolic pathways. Existing gene expression studies have primarily addressed cold acclimation responses in herbaceous plants, and few have focused on perennial evergreens, such as conifers, that survive extremely low temperatures during winter. Relative to Arabidopsis leaves, the main transcriptional response of Norway spruce (Picea abies (L.) H. Karst) needles exposed to cold was delayed, and this delay was associated with slower development of freezing tolerance. Despite this difference in timing, our results indicate that, similar to herbaceous species, Norway spruce principally utilizes early response transcription factors (TFs) of the APETALA 2/ethylene-responsive element binding factor (AP2/ERF) superfamily and NAM (no apical meristem)/ATAF (Arabidopsis Transcription Factors)/CUC (cup shaped cotyledon) (NACs). The needles and root of Norway spruce showed contrasting results, in keeping with their different metabolic and developmental states. Regulatory network analysis identified conserved TFs, including a root-specific bHLH101 homolog, and other members of the same TF family with a pervasive role in cold regulation, such as homologs of ICE1 and AKS3, and also homologs of the NAC (anac47 and anac28) and AP2/ERF superfamilies (DREB2 and ERF3), providing new functional insights into cold stress response strategies in Norway spruce.

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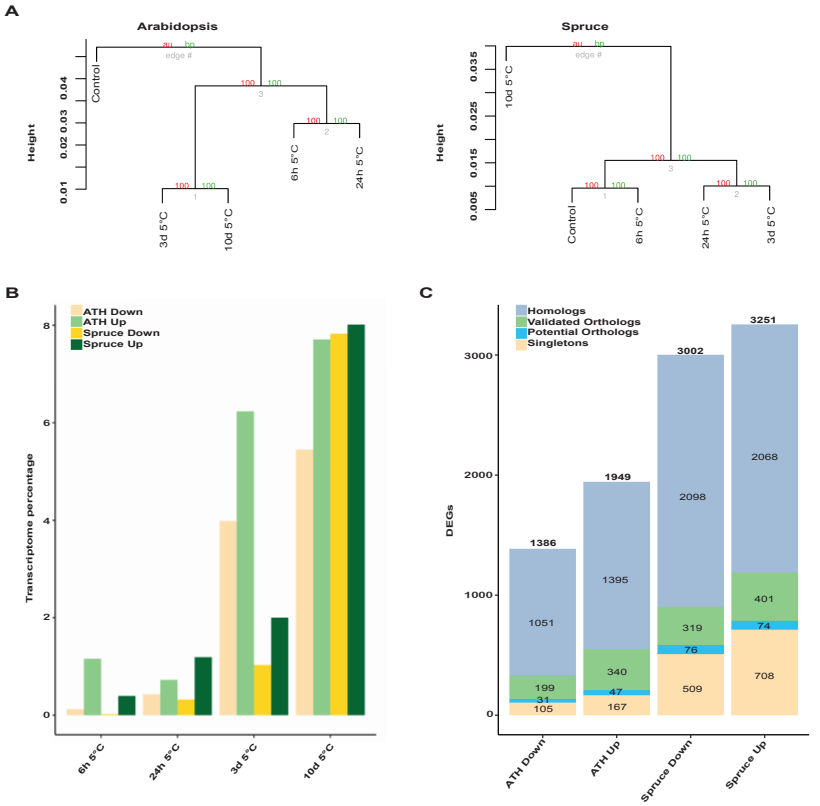

Figure 1. Comparing the response of *Arabidopsis thaliana* leaf and *Picea abies* (Norway spruce) needle transcriptomes exposed to 5°C. A) Hierarchical clustering using normalized data (see methods). The red numbers correspond to Approximately Unbiased (AU) values and the green ones to Bootstrap Probability (BP) values. **B)** Analysis of transcriptome progression in response to cold. Differentially expressed gene lists (DEGs) were obtained at each point in the time series, compared against the control, and then represented as a percentage of the transcriptome. DEGs significantly induced in Arabidopsis (green) and Spruce (dark green) and significantly repressed DEGs in Arabidopsis (yellow) and Spruce (dark yellow) were obtained by filtering the data by corrected Pvalue ≤0.01 and Fold Change ≥2. **C)** Orthologs, Homologs and Species-specific DEGs for both species (down and up-regulated). Validated orthologs correspond to orthologous genes that are differentially regulated by cold in both species. Gene lists for each group and functional information are available in Supplemental Table S1.

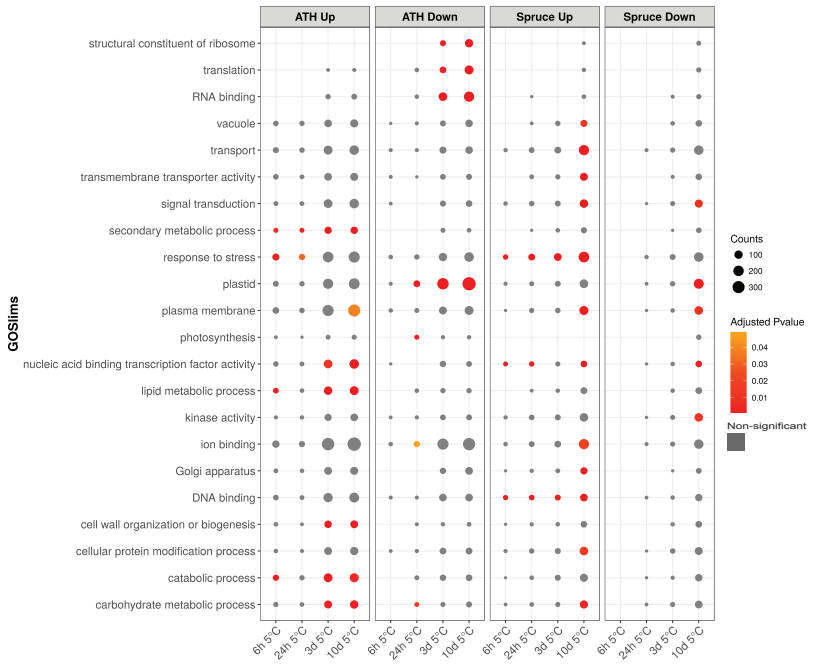

Figure 2. GOSlim functional analysis of species comparison. Aerial tissues transcriptomic responses of Arabidopsis thaliana (ATH) and *Picea abies* (Spruce) to chilling (5°C) were compared functionally by GOSlim analysis. Differential expressed genes (DEGs) lists along time series treatments with different GOSlim tags assigned in the up and down-regulated DEGs were analysed. Amount of genes of each category are represented by bubble sizes (counts). GOSlim enrichments with Bonferroni corrected P-values ≤0.05 are represented with a red colour scale. Non-significant P-values (corrected P-values >0.05) are in grey. Here only a selected group of GOSlim categories are represented. A full list including all the categories and P-values is included in Supplemental Table S4 and a description of the genes and their homologs in Supplemental Table S5.

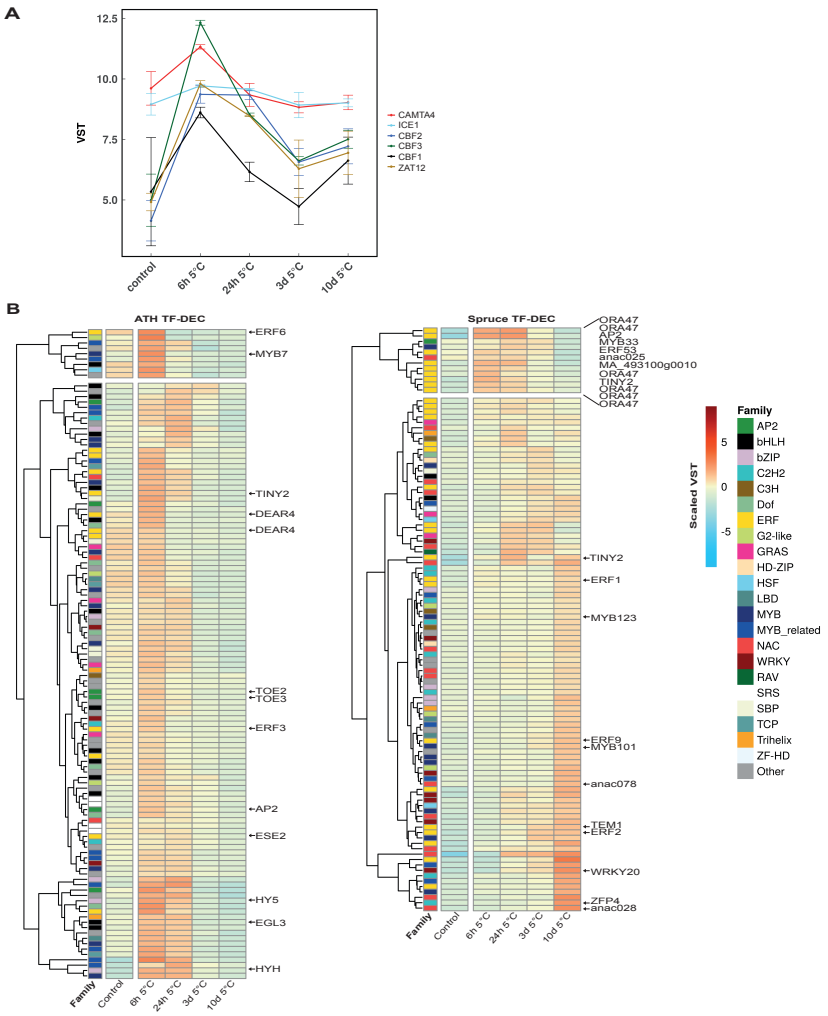

Figure 3. Transcription factor analysis. A) Expression profiles of previously characterized Transcription factors (TF) involved in the cold response in *Arabidopsis thaliana: CAMTA4* (AT1G67310), *ICE1* (AT3G26744), *CBF1* (AT4G25490), *CBF2 (*AT4G25470), *CBF3* (AT4G25480) and *ZAT12* (AT5G59820) are shown using variance stabilizing transformation (VST) gene expression values from our experiment (n=3). Errors bars represent SD. **B)** TF differentially expressed by cold (TF-DEC) were analyzed in both Arabidopsis and Norway spruce. TF with positive changes relative to control are shown (corrected P-value ≤0.01 and Fold Change ≥2). VST data were scaled by row means. For each heatmap zoom versions including all the identifiers are available in Supplemental Fig. S1 and S2.

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`Fig4.pdf` available at https://authorea.com/users/438787/articles/539898-norway-spruce-deploys-tissue-specific-responses-during-acclimation-to-cold

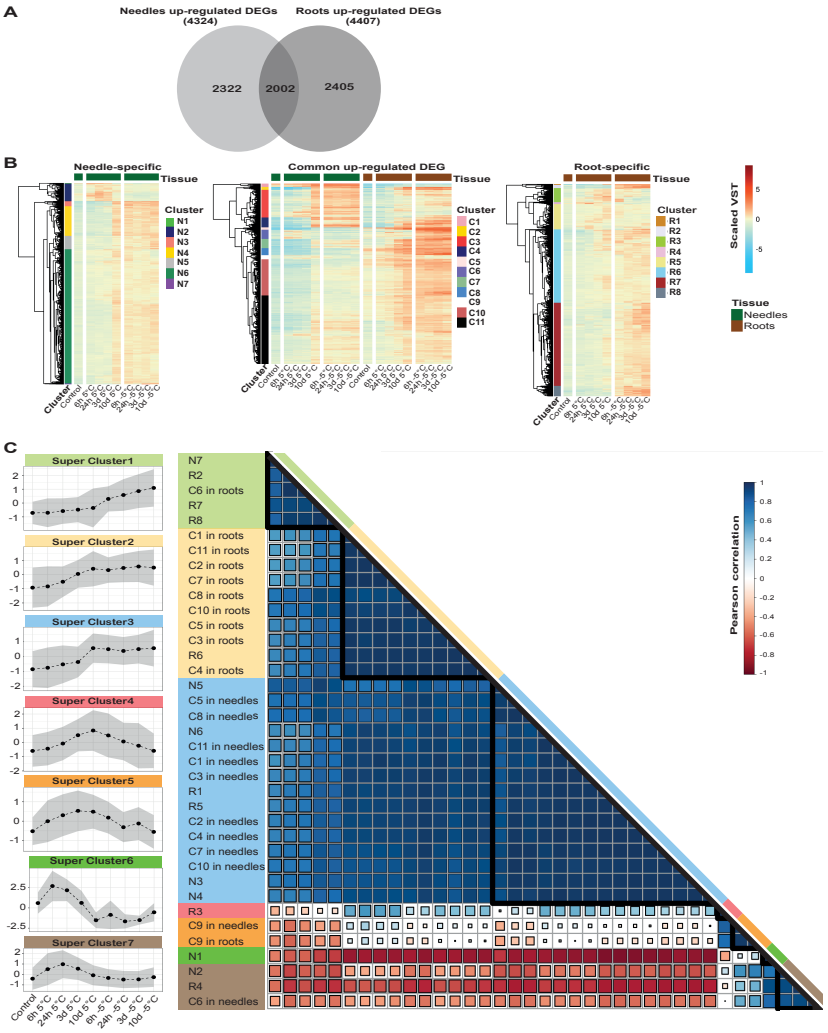

Figure 5. Identification of coordinated responses to cold. A) Venn diagram representing needle-specific, root-specific and common up-regulated differentially expressed genes (DEGs). A Gene Ontology (GO) enrichment analysis of these gene lists is available in Supplemental Table S8. **B)** Heat maps and main clusters of tissue specific and common up-regulated DEGs. Common DEGs were analyzed on both tissues and each identified cluster (C) was separated according clusters trends in needles (N) or in roots (R). **C)** Super clusters (SC) were defined by comparing clusters from Fig. B using Pearson correlation analysis. Common clusters (C) were separated by tissues to compare against tissue-specific clusters. So a cluster trend for a common cluster number *"i"* was called 'C_i in needles' or 'C_i in roots' according the tissue analyzed. For each SC, scaled gene expression mean values are represented with dotted lines. Grey areas represent variability by two standard deviation. An analysis of SC distribution in the network is available in Supplemental Figure S3. A GO enrichment analysis for these SC is available in Supplemental Table S9.

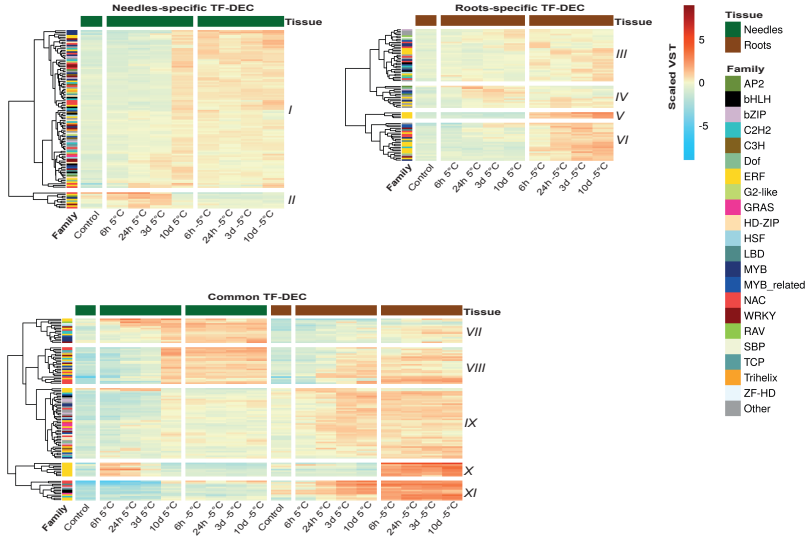

Figure 6. Transcription factors differentially regulated by cold (TF-DEC). TF-DEC were analysed by heat maps and hierarchical clustering (corrected P-value ≤0.01 and Fold Change ≥2) crossing normalized needles and roots gene expression data. Families members were obtained from Plant Transcription Factor Database (Jin, Zhang et al. 2014) and normalized VST expression were scaled by row means. A file including gene descriptions for each cluster is available in Supplemental Table S10.

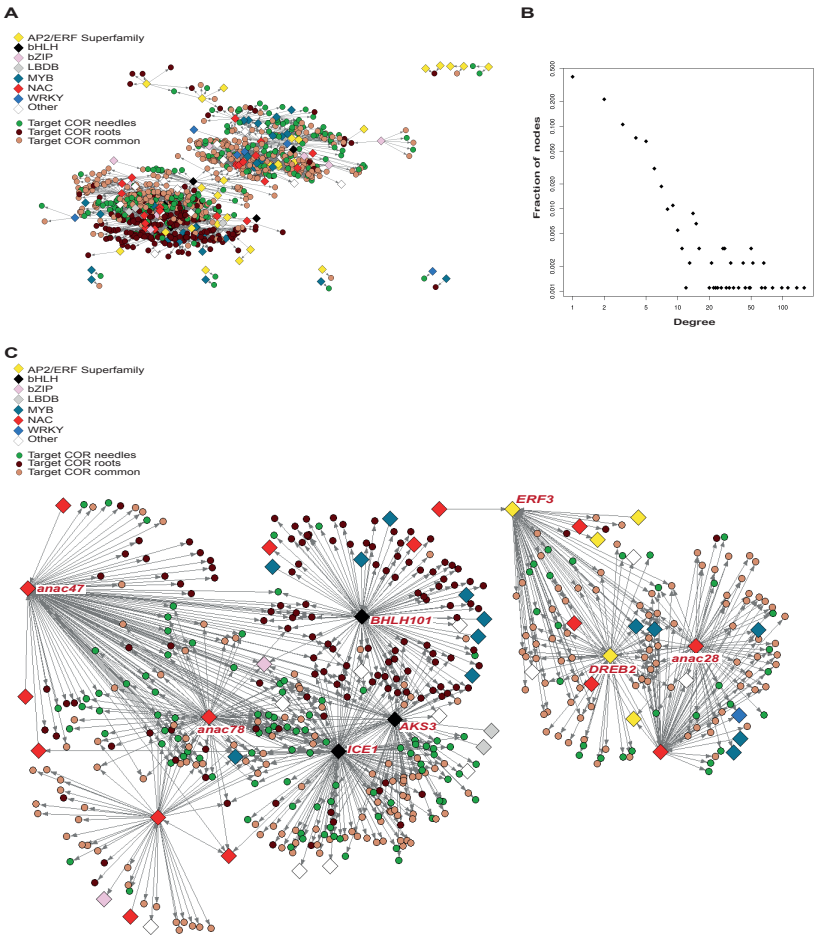

Figure 7. Regulatory network analysis. A) Network representation of predicted regulatory interactions between Transcription Factors (TF) and cold responsive (COR) genes. TFs are represented by diamonds and their family by colors. COR genes are represented by circles and colored according to the tissue in which they are differentially regulated. **B)** Network Degree distribution in Log10/Log10 scale. **C)** Sub-network of the 10 genes with the highest centrality. Gene Ontology (GO) enrichments in the hub neighborhoods are available in Supplemental Table S12 and topology information and gene aliases are available in Supplemental Table S13.