# Poplar as a Tree Model for Horticulture and Beyond: a Case Study of Genome-Scale Changes in Gene Expression during Bud Entry and Release from Dormancy

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**Abstract**

**With the available genomic sequence and other experimental attributes, poplar has emerged as a leading candidate to study traits specific to woody perennial plants. Genomic and molecular knowledge gained from studies related to flowering, stem development, tree architecture, phenology and dormancy can be extended readily to woody species in forestry and horticulture. As an example of the value and transferability of poplar genomic studies to tree biology, we conducted a genome-wide transcript analysis study utilizing a NimbleGen microarray representing 65,966 individual gene sequences derived from the poplar genome sequence. The investigation included more than 20 tissue types and several developmental sequences. Presented here is a preliminary analysis of changes in gene expression during entry into and exit from bud dormancy. Potential applications of these data include identification of candidate genes for studying natural polymorphisms, targets for reverse genetic studies, target promoters for control of gene expression, and tools for many forms of marker-aided breeding and genetic engineering of woody plant growth and development.**

## INTRODUCTION

Seasonal bud dormancy is critical for survival and productive growth of perennial plants. Similar to other perennial plants, shortening day lengths and reduced temperature during autumn provides environmental cues to begin dormancy induction in buds. This involves the slowing of meristematic activity, development of surrounding bud scales, and the acquisition of dehydration and cold tolerance. We analyzed the transcriptome changes that occur in whole vegetative buds during entry, maintenance, and near to exit from dormancy using a Nimblegen whole genome microarray representing 65,966 individual poplar sequences including nuclear, organelle and microRNA precursor genes. We report that at least 5000 genes show differential regulation during this period.

## MATERIALS AND METHODS

Lateral buds of rapidly growing, three-year-old *Populus trichocarpa* trees (clone Nisqually-1) at a field site in Corvallis, Oregon (USA) were sampled between August 2005 and March 2006. The tissues from the initial sampling point had immature bud scales covering the whole bud (August). The next 3 stages (November, December, and February) also included bud scales and represented subsequent stages of bud development. The last sampling point (March) was two weeks prior to the start of visible bud flush.

RNA from vegetative buds were isolated using Qiagen RNeasy kit following the manufacturers protocols, incorporating the Qiagen "on column" DNase I treatment to remove contaminating genomic DNA. Quality of RNA was tested using an Agilent Bioanalyzer, labeled and hybridized to microarrays for expression profiling. The array was a custom poplar Nimblegen microarray (http://www.nimblegen.com/) targeting 55,794 predicted transcripts from the *Populus trichocarpa* genome sequencing project, 126 mitochondrial and chloroplast gene models, and 9,995 unigenes derived from aspen



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EST sequences (Sterky et al., 2004). Array hybridization, quality control, and data extraction were carried out by Nimblegen using their established microarray processing pipeline (Wang et al., 2006). Microarray data were normalized across all experimental arrays using the Bioconductor – Robust Multiarray Averaging (RMA) protocol. Gene Spring 7.3 (Agilent Technologies, USA), EDGE (Leek et al., 2006), Microarray Software Suite-MeV4.0 (http://www.tm4.org/), and Cytoscape (http://www.cytoscape.org/) software were utilized for further data analysis and graphical display of results.

## RESULTS AND DISCUSSION

When the five bud developmental stages were considered, there was detectable expression for more than 44,000 of the 65,915 transcripts represented on the array in at least one stage. EDGE indicated that more than 7,000 *Populus trichocarpa* transcripts were differentially expressed within the bud development series (Q, false discovery rate, cutoff of 0.01). More than 5,000 of these genes were regulated greater than 2-fold when the highest and lowest expressing stages were compared.

The clustering of all regulated genes (Fig. 1) illustrates the major patterns of gene expression during the dormancy cycle. A large group of genes (Fig. 1, top half) showed down-regulation during entry into dormancy. The majority of genes within this group stayed inactive during the sampling period, however, a small proportion of genes was reactivated during exit from dormancy. In contrast, another cluster of genes (Fig. 1, lowest part of figure), showed activation only during exit from dormancy (February, March). The cluster of genes circled in Figure 1 were induced during November and December and were down-regulated during exit from dormancy (February, March). Gene Ontology category analysis illustrated an enrichment of genes associated with stress responses, transporter activity, catalytic activity, transcriptional regulation, and cell membrane related genes in this group (Fig. 2). It included carbohydrate metabolic genes, dehydrins, LEAs, MAP and Ser/Thr kinases, water and cold stress induced proteins, ARFs, ethylene and ABA related proteins, and several classes of transcription factors.

Out of the 2,576 transcription factors and regulatory genes in two poplar databases (http://dptf.cbi.pku.edu.cn/ and http://plntfdb.bio.uni-potsdam.de/v2.0/), more than 400 differed greater than two-fold between sampling points. More than half of the regulated transcription factors were down-regulated during onset of dormancy. However, there was another group of transcription factors that were activated during the onset of dormancy including members belonging to ZF, MYB, MADS, AP2-EREBP, WRKY, and NAC classes. Poplar homologues of cherry, rice and Arabidopsis CBF/DREB AP2 transcription factors involved in the cold response pathway, many of which have been identified as critical for cold acclimation, showed rapid induction beginning in our November samples (Fig. 3).

CAZymes (Carbohydrate Active enZymes) (http://afmb.cnrs-mrs.fr/CAZY), which include families of glycosyl transferases (GTs) and glycosyl hydrolases (GHs), showed major reconfigurations during dormancy (Fig. 4). The expression of GTs and GHs showed distinct clusters of genes that were associated with onset of dormancy. These genes may be associated with carbohydrate metabolic changes that promote the development of cold tolerance or facilitate carbohydrate storage and mobilization.

## CONCLUSIONS

More than 5,000 genes, including almost 400 transcription regulators, showed at least two-fold differential regulation during bud dormancy and release. Clustering identified groups of genes, including those encoding numerous transcription factors, which were co-regulated during different phases of the dormancy cycle. Genes related to stress response, carbohydrate metabolism and transporter activity showed very active regulation during dormancy.

**Literature Cited**

Leek, J.T., Monsen, E. et al., 2006. EDGE: extraction and analysis of differential gene expression. Bioinformatics 22:507-8.

Sterky, F., Bhalerao, R.R. et al., 2004. A *Populus* EST resource for plant functional genomics. Proc. Natl. Acad. Sci. USA 101:13951-6.

Wang, X., He, H. et al., 2006. NMPP: a user-customized NimbleGen microarray data processing pipeline. Bioinformatics 22:2955-7.

**Figures**

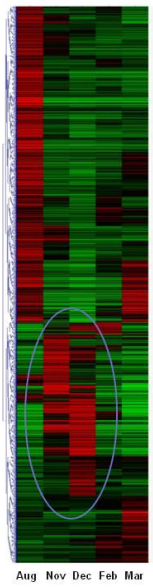


Fig. 1. Hierarchical clustering of all differentially expressed genes during dormancy cycle. (green = downregulated, red = upregulated).

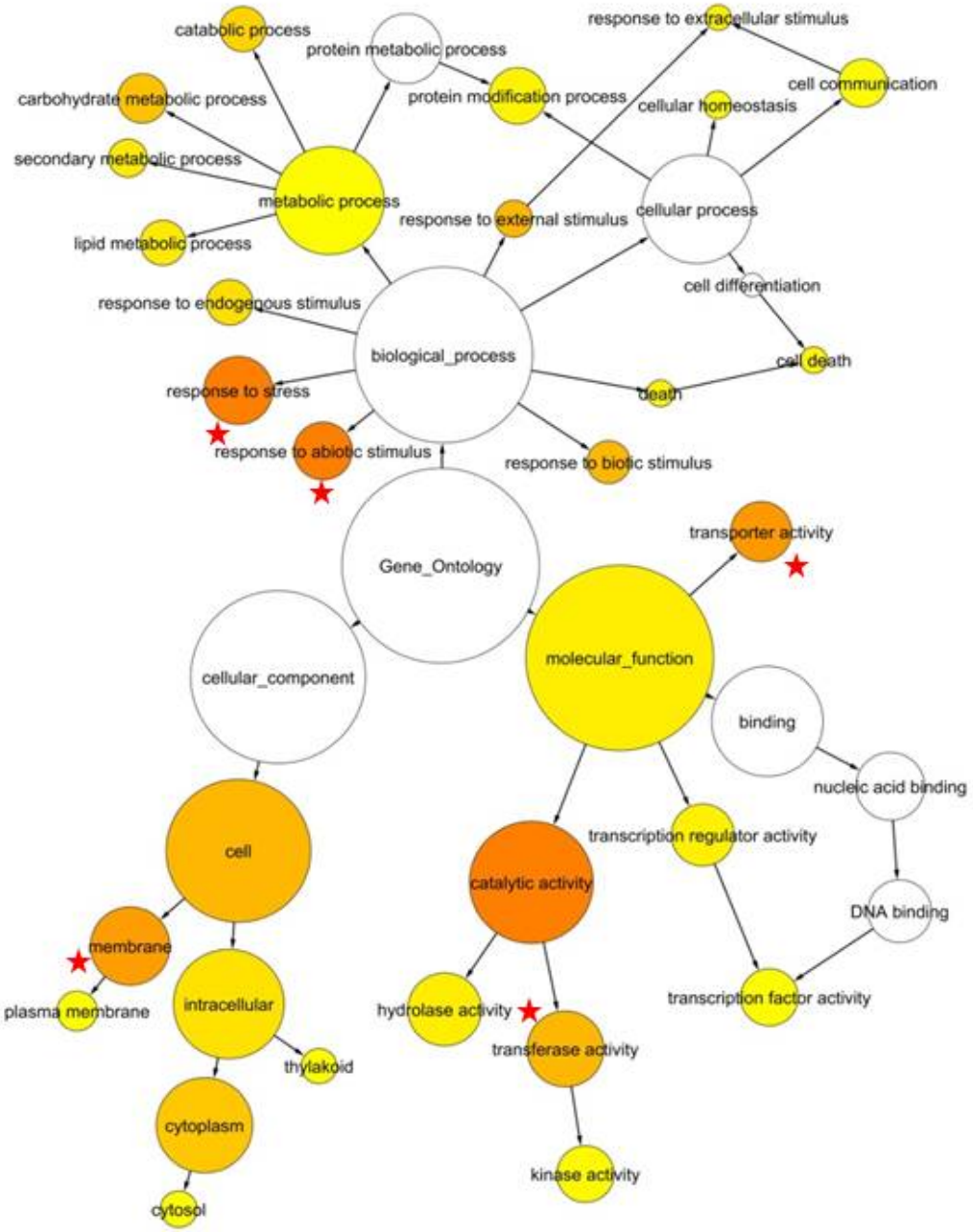


Fig. 2. Over-represented gene ontology categories during dormancy. The intensity of color represents level of statistical significance. Stars represent highly over-represented categories.

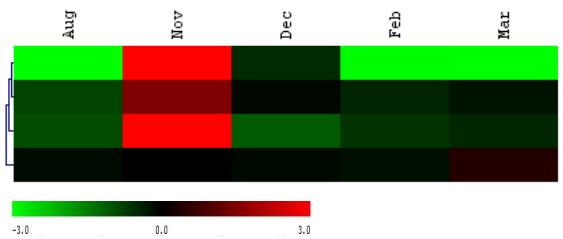


Fig. 3. Expression of four poplar homologs of cherry, rice and *Arabidopsis* CBF/DREB AP2 cold response pathway transcription factors. (green = downregulated, red = upregulated).

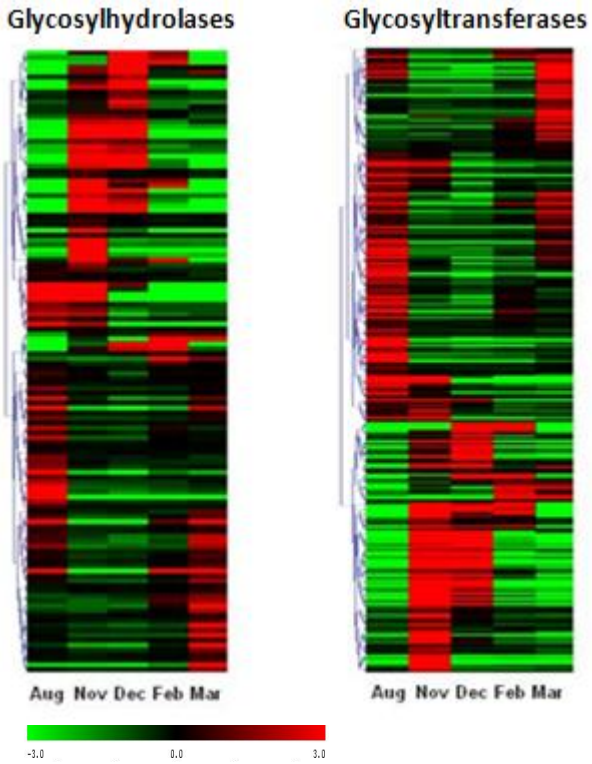


Fig. 4. Cluster diagrams of expressed carbohydrate catalyzing gene family members (glycosyltransferases and glycosylhydrolases).