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Growth-phase-dependent gene expression profiling of poplar suspension cells

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Objectives

We have analyzed the global pattern of growth-phase-dependent gene expression of poplar suspension cells during in vitro culture using a high-density cDNA microarray.

Materials and Methods

1. Plant Material- *Populus alba* x *P. tremula* var. *glandulosa*

2. Methods: Poplar suspension cells were subcultured by transferring 0.4 g (fresh weight) of cells to 100 ml liquid MS medium containing 1 mg l^{1} 2,4-D, 0.1 mg l^{-1} NAA and 0.01 mg l^{-1} BAP. The suspensions were maintained at 100 rpm on a gyratory shaker in the culture room at 221 C under 20 mol $m^{2}s^{-1}$ with cool white fluorescent light. For target RNA preparation, cells were harvested every two days after subculture until 30^{th} days by vacuum filtration through a couple of 3MM filter papers, weighed, frozen in liquid nitrogen, and kept at -80C. Microarray data acquisition and analysis were performed using GeneSpring 7.2 (Silicon Genetics, Redwood, CA).

Results and Discussion

On the basis of 8,964 expressed sequence tags of poplar, obtained from a cDNA library of poplar suspension cells, we have created a DNA microarray consisting of 3,378 unique clones. Here we have used the microarray to study gene expression profiles during normal growth kinetics of poplar suspension cells. The level of RNA transcript at 12 time points covering whole growth phase was compared with the level of transcript at common reference consisting of a pool of RNAs from all 12 time points. A total of 972 genes (24.5%) were shown to be differentially regulated by a minimum of twofold over the time course. The subtle expression patterns observed in the 972 genes were reduced to five major patterns of expression. Cluster analysis of the expression profiles highlighted a major switch in gene expression at the early log phase. The reliability of the expression profiles obtained with the arrays was proven by northern blot analysis with 30 selected genes.

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