TARGETING ACETYLATION IN ASPEN (*P. tremula * P. tremuloides*)

Vimal Kumar B

Plants are sessile organisms, which developed a rigid cell wall to prevent them from adverse effects of abiotic factors around them. Plant cell wall consists of various types of polysaccharides, which are held together by strong and complex chemical bonding. Though a lot of progress has been made in understanding the cell wall biosynthesis process, still much need to be made to understand the biosynthesis of different cell wall polymers and their interaction in cell wall. Almost all polysaccharides except cellulose are acetylated to various degrees and acetylated hemicellulose forms major part of biomass in secondary cell wall of wood.

Secondary cell wall found is considered to be a good source for renewable energy fuel since it forms large part of biomass of wood part of trees. A major hurdle in cost effective fuel production from lignocellulosic feedstock is the recalcitrance due to the presence of acetyl groups present in many of the cell wall polysaccharides. These acetyl groups released during pretreatment create an acidic environment that inhibits the growth and activity of most of the hydrolyzing enzymes.

Genetic approaches were used in *Arabidopsis thaliana* to elucidate acetylation pathways by finding respective genes involved in acetylation. Mutation studies were also carried out with them to reduce acetylation levels of various polysaccharides present in the cell wall. As mutation study is not possible in trees like aspen, RNAi strategy was used to reduce the mRNA level of acetyl transferases genes thereby obtaining plants with reduced acetylation and also to study the actual function of acetylating genes. RNAi approach with two different promoters (35S promoter and "Wood specific" promoter) has been adopted to reduce the transcript level of a gene family that included four members with predicted function in cell wall acetylation. In my project work qPCR was used to analyze the mRNA level of acetyl transferase genes in mutant trees in comparison with the wild types. The mutant lines showing efficient down regulation of wood secondary cell wall acetylation genes were identified.

Also another approach to reduce the acetyl content by over-expressing fungal enzyme, which specifically remove the acetyl groups present in the cell wall polymers, has also been used to create transgenic aspen lines. I analyzed the mRNA level of overexpressed fungal gene in transgenic aspen trees in comparison with the wild types. The mutant lines showing significant up regulation of fungal genes were identified. These mutant lines from two projects will be studied for improved saccharification process.

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Biology Education Centre, Uppsala University, and Department of Forest Genetics, SLU, Umeå
Supervisors: Ewa Mellerowicz and Prashant Mohan Pawar