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Poster Abstract – D.26

CIS -ACTING REGULATORY VARIATION IN WHITE POPLAR

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Poplar is a model species in forest trees whose genome has been fully sequenced and is an important source for the production of timber, plywood, pulp and paper.

Functional polymorphisms in or around genes can be classified as coding variation, altering the amino-acid sequence of the encoded protein, or regulatory variation, affecting the level or the pattern of expression of the gene. While the frequencies and consequences of coding polymorphism can be recognized directly from the DNA sequence, the extent to which variations in non-coding *cis*-regulatory DNA alters gene expression in populations is mostly unknown. It has been suggested that regulatory variations are important in modulating predisposition to disease susceptibility, and to be the primary substrate for the evolution of the species. Studies in maize and *Arabidopsis* demonstrated that the *cis*-acting regulatory variation is a widespread phenomenon, and that is also relevant to abiotic stress response.

We set out a project to estimate frequencies and magnitudes of *cis*-acting regulatory variation in *P. alba.*

Particular attention was focused on genes involved in the mechanism of UV-B stress tolerance.

To detect poplar *cis*-acting regulatory variation we used a method that has been developed and widely used in our lab in maize. The method involves the study of two alleles of a gene in heterozygous individuals and the comparison of the transcript expression associated with each allele. This allows recognition of *cis*-acting variation without the identification of specific regulatory variants (which can be hundreds or even thousands of bases upstream from the transcription unit). SNP markers (Single Nucleotide polymorphisms) in the transcript itself are used to distinguish between transcripts derived from one of the two alleles.

To study the genes involved in UV-B stress resistance we isolated RNA from the petiole-induced calli after UV-B treatment, as well as from their respective controls. The analytical method involves RT-PCR amplification of the region surrounding the SNP marker, Single Base Extension (SBE) of a primer adjacent to the variant base in the presence of fluorescently labeled nucleotides and detection on a capillary DNA sequencer. The ratio of the two alleles is inferred by comparison with known mixtures of homozygous genomic DNA mixes used as reference standard, which, after PCR amplification, are subjected to the same SNP assay.

This type of study will help us not only to appreciate the extent of functionally important regulatory variation but also to focus on candidate haplotypes with differential expression in order to characterize specific polymorphisms.