

P4–TREE GENOMICS

Organised by G. Taylor for the Plant Environmental Physiology Group and sponsored by The Forestry Research Coordination Committee

P4.1–The Populus sequencing effort: From draft assembly to functional annotation

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Abstract not supplied

P4.2–The Swedish populus genome project

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The Swedish Centre for Tree Functional Genomics, consisting of groups at Umeå Plant Science Centre and the Royal Institute of Technology, Stockholm, have sequenced and annotated over 100 000 ESTs from 19 different cDNA libraries from *Populus tremula x tremuloides*, *Populus tremula* and *Populus trichocarpa*, produced from different tissues in different developmental stages and subjected to different stresses. The assembly of these ESTs indicated that they represent almost 25 000 different genes although the true number might be between 15 and 20 000. About 75% of the *Arabidopsis* have however a very significant hit ($E < 10^{-10}$) in our database. We have produced over 1000 spotted DNA microarrays consisting of 13 488 sequences (verified by 5'- and 3'- resequencing) and are in the process of constructing the second generation of Populus arrays, based on the Unigene set of the 100 000 ESTs. These microarrays are currently used for global transcriptomics in diverse tree biology research areas, such as wood formation, leaf development, leaf senescence, dormancy, biotic and abiotic stresses and transgene characterization. Access to the arrays is possible on a collaborative basis. Our global gene profiling approach is linked to protein and metabolite profiling through large-scale proteomics and metabolomics. Our vision is that this genomic approach will allow an unprecedented and comprehensive insight into gene expression correlated with the amounts and types of proteins and metabolites present in specific tissues. A large number of genes involved in wood formation will also be put into a high throughput knockout program.

P4.3–Tree genes in future climates: Global gene expression and long-term adaptation of trees to elevated atmospheric CO₂

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Over half of all global carbon held in vegetation is in forests. The consequences of increasing atmospheric carbon dioxide for the functioning of forest ecosystems remain uncertain although it has been suggested that 'long-term' adaptation to increased CO₂ will negate growth-stimulations that occur in the short-term, limiting the potential of forests to sequester additional carbon in future climates. Given the lifespan of trees, gene-level adaptation to altered environments will determine the potential for such sequestration and will also be central to patterns of development, function and interactions with biotic and abiotic stresses. Here we show that in genome-wide expression of >10 000 EST (expressed sequence tags), representing partially sequenced genes, following long-term exposure of a poplar forest to elevated CO₂ results in approximately 860 (7%) genes being significantly up-regulated, and 480 (4%) genes being significantly down-regulated. In contrast, for genes determining photosynthetic fixation of carbon, 26% of ESTs are down-regulated in elevated CO₂. A large and previously unobserved down-regulation of the light harvesting machinery for photosynthesis was also quantified. Of particular interest was the consistent up-regulation of genes associated with the auxin and ethylene biosynthetic pathway, suggesting these hormones play a key role, previously undocumented, in determining long-term developmental responses to elevated CO₂. We conclude that gene-level adaptations are likely to be important in optimising tree growth in future CO₂ atmospheres and that for fast growing bioenergy trees, continued positive growth responses in future CO₂ climates seem likely.

P4.4–Functional genomic dissection of vascular development in higher plants

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We have exploited the extensive genomic resources in *Arabidopsis* and the model tree, hybrid Aspen to identify

several novel regulators of vascular development. At least 2 candidate regulatory sequences have been identified using a reverse genetic strategy in *Arabidopsis* that target orthologs of poplar sequences expressed in the vascular cambium. In parallel, an innovative forward genetic screen has identified at least 4 loci that disrupt vascular patterning in germinating *Arabidopsis* seedlings. We are probing their developmental defect(s) using a unique panel of vascular markers in conjunction with confocal microscopy. These novel tools and approaches will provide a genetic framework for vascular development.

P4.5—Exploring biodiversity under a latitudinal gradient: morpho-Physiological behaviour and molecular genetics in *Populus Alba*

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Abstract not supplied

P4.6—Functional genomics of terpenoid defenses in spruce

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Terpenoid (isoprenoid) metabolites form the predominant chemical defense system of many conifer species. These chemicals serve to protect trees against a plethora of potential herbivores and pathogens. The conifer terpenoids are most prominent as resin components (mainly monoterpenes and diterpene resin acids), as constitutive volatiles including monoterpenes and the hemiterpenes isoprene or methyl butenol, as insect juvenile hormone analogues, or as induced volatile alarm signals that function in tritrophic plant–insect interactions. My group in the UBC Biotechnology Laboratory together with the Genome BC/Genome Canada Forestry genome project has applied a combination of large-scale EST mining, targeted gene discovery, biochemical enzyme identification, and metabolite profiling to characterize terpenoid defenses in species of spruce. Our studies include Norway spruce (*Picea abies*), White spruce (*P. glauca*), and Sitka spruce (*P. sitchensis*) as well as their hybrids. Mining of more than 30 000 ESTs and targeted library hybridization yielded cDNAs for most of the enzymes in conifer terpenoid biosynthesis. These include enzymes of the MEP- and mevalonic acid pathways, prenyl transferases, a family of functionally diverse terpene synthases, and cytochrome P450-dependent enzymes. Gene expression profiles were monitored in response to mechanical wounding of trees,

treatment with methyl jasmonate, and insect feeding. Induced expression of terpenoid defense genes is associated with cell differentiation of traumatic resin ducts in developing xylem and de novo emission of signal molecules of plant–insect interactions.

P4.7—Selection and breeding of conifers in Britain and the scope for marker aided selection

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Forest Research is about to embark on a programme of Marker Aided Selection for its principal conifer species—Sitka spruce (*Picea sitchensis*). The overall objective is to increase the efficiency of Sitka spruce breeding and selection by screening in the laboratory for economically important traits relating to quality and volume. The initial objective is to find markers for wood density although it is expected that the programme will be extended to include a large number of markers relating to a whole spectrum of traits. Screening with markers will improve the gains available to the forestry industry by increasing the selection intensity and identifying at a very early age those rare genotypes that combine generally conflicting traits such as fast growth rate and high wood density. Cryopreservation and somatic embryogenesis will assist in delivering the gains to industry. The first step of the new programme is to establish an accurate relationship between phenotypic field performance and markers identified in the laboratory. A large clonal trial is planned which will be replicated across climatically contrasting sites. Trees for this trial (1500 genotypes from each of 3 full-sib families; 4500 genotypes in all) are currently being raised in the nursery for field planting in spring 2005.

Work has started on selection of the most suitable form of molecular marker—thought to be either AFLPs or SSR. Marker work will commence soon with the establishment of a molecular map for one of the full-sib families.

P4.8—Environmental genomics in forest trees as exemplified by drought stress in pine, dormancy related traits and hypoxia in oak

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Growth, development and productivity of forest trees are continuously challenged by abiotic stresses and may also

be greatly affected by rapid climatic changes in the near future. If they have to survive without having to migrate and colonize new habitats, these long-lived organisms will have to develop particular strategies of adaptation allowing them to cope with changing environmental conditions. However, because of a predicted increasing rate of global change, there is a risk of reduced potential for long-term adaptation comparing to annual plants with much higher reproduction rate. One question is therefore whether the present structure and amount of genetic diversity in tree species is sufficient enough to be sustainable in the long-term and allow adaptation to future stressful conditions. Answering this question will help undertake actions to preserve adaptability and avoid major losses. In this context, we have taken a three-phase approach to study the genetic, physiological and molecular mechanisms underlying forest tree adaptation and response to natural selection, focusing on three adaptive traits: drought tolerance, dormancy and root hypoxia in pine and oak. In phase one, we estimate the genetic variance and heritability of the traits and locate their mendelian inherited components (i.e. QTLs) on genetic maps. In phase two we use a gene discovery approach to identify and characterize the genes and proteins involved in adaptation. In phase three we are developing a candidate gene approach to relate the variability of candidate genes with the variation of the traits within pedigree and in natural populations.

P4.9—Genetic Mapping of Important Agronomic Traits in Biomass Willows

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Increased energy production from renewable sources is currently a high priority in Europe in response to the growing need to replace fossil fuels in ways that are non-polluting. Fast-growing biomass crops, such as short rotation coppice (SRC) willows, contribute to the solution. In order to meet future European Union targets for additional biomass energy production, the breeding of high yielding, pest- and disease-resistant SRC crops urgently needs to be accelerated. To underpin such breeding efforts, we have embarked upon a programme of research aimed at providing molecular markers linked to key agronomic traits for use in marker-assisted selections. Towards this goal, we have used AFLP and microsatellite markers to construct two genetic linkage maps of willow based on the mapping populations K3 and K8, comprising 66 and 947 individuals respectively. Ongoing laboratory and field-based phenotypic assessments indicate that important traits, such as resistance to *Melampsora* rust, resistance to willow beetle herbivory and a number of yield parameters, are segregating in these populations. By using both genotype and phenotype data in QTL analyses, a number of genomic regions

linked to target traits have now been identified and are currently undergoing more detailed study.

P4.10—QTL discovery in biomass poplar grown in short rotation

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Fast-growing *Populus* hybrids grown under short rotation coppice (SRC) for biomass may help to meet escalating demands for renewable fuels and to off-set emissions of CO₂ in line with the Kyoto protocol. It is, therefore, important to identify and map the quantitative traits that make an ideal tree for short rotation coppice, and to look at the possibility of incorporating these traits into commercial poplar by marker aided selection. Several cell, leaf and branch traits have been identified which may contribute to an ideotype for yield in SCR poplar. A three-generation interspecific pedigree of *P. trichocarpa* x *P. deltoides* with over 350 F₂ individuals is being used to study such traits, and correlations between traits are being studied to identify those that are important. Correlations have been found between yield measurements such as mass, height and basal diameter with cell, leaf and branch traits, indicating some of the traits which cause an overall increased biomass.

Microsatellite and AFLP markers have been used to genotype 300 F₂ individuals from the interspecific pedigree to produce an enriched molecular linkage map. This enhanced map is being used to look more closely at QTL for improved yield and other important correlated traits leading to a better understanding of biomass at a genetic and cellular level, and with the expectation of accelerated breeding to improve SRC Poplar.

P4.11—An integrated approach to mapping in *Rubus*

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Red raspberry belongs to the genus *Rubus*, of the *Rosaceae* family, an economically important family of perennial fruit bearing crops that includes members of the following genera: *Malus* (apple), *Pyrus* (pear), *Rubus* (raspberry), *Fragaria* (strawberry) and *Prunus* (stone fruits). In addition the family includes a number of important ornamentals such as Roses, flowering cherry, crabapple and quince. As well as its commercial and nutritional importance, red raspberry is an ideal scientific model for the development and application of genomic technologies in perennials, particularly the *Rosaceae*. Characterised by a short maturation period and a small (0.3 pg, 270 Mbp) diploid genome make it a logical candidate for genetic and physical mapping,

large-scale sequencing and comparative mapping. A phenotypically well-characterised genetic linkage map of red raspberry based on 410 SSRs and AFLP markers has been generated. A nebulised small insert genomic library is currently being sequenced to allow us to estimate gene density. Large insert genomic libraries (BACS) are being constructed to serve as a source of genomic DNA for physical mapping, positional cloning

and as a scaffold for whole genome sequencing. Anchoring the physical map to the genetic map would enable alignment of the maps and facilitate the identification of genomic regions harbouring genes controlling important phenotypes. An integrated physical/genetic map will also allow the extent of synteny or collinearity of the *Rubus* genome with other members of the *Rosaceae* to be determined.