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FOREST BIOTECHNOLOGY '99—A JOINT MEETING OF THE INTERNATIONAL WOOD BIOTECHNOLOGY SYMPOSIUM AND THE IUFRO WORKING PARTY FOR MOLECULAR GENETICS OF TREES, 11–16 JULY, 1999, OXFORD, UK†

D. ELLIS[1*] and T. STRABALA[2]

[1]*BC Research, Inc., Vancouver, B.C., Canada;* [2]*Genesis Research and Development Corp. Ltd., Auckland, New Zealand*

What better way to keep oneself in perspective when attending the premier forest biotechnology meeting of 1999 than to walk each day into a huge glass-roofed edifice, one of the finest examples of neo-Gothic architecture in England, only to be towered over by the ancient skeletal remains of an iguanodon, an enormous Cretaceous dinosaur. Among the other dinosaur skeletons and eggs one had to pass on the way to the conference room was a display which served as an inspiration to Lewis Carroll for *Alice in Wonderland* containing the most complete remains in existence, a head and foot, of the now extinct dodo. The Oxford University Museum of Natural History, site in July 1860 of the infamous Wilberforce and Huxley debate on creation versus evolution, was also the site of *Forest Biotechnology '99* almost 140 years later.

The aim of the meeting was to provide a forum to present the most current advances in the application of molecular biology and genetics to forest trees. Over 200 scientists from 30 countries shared information focused on forest tree species covering a wide range of topics, including *in vitro* culture, genetic engineering, molecular analysis of developmental, adaptive and physiological traits, cell wall biosynthesis and modification, and genomics and genome mapping. Like the evolution debate of the nineteenth century, this meeting produced considerable debate and some external attention because, in addition to the 'traditional' scientific presentations, the meeting played host to an anti-biotechnology presentation and was also the site of an anti-biotechnology protest organized by Genetically Engineered Free Forests (GEFF), a group assembled specifically in response to the meeting. In addition, on the opening night of the meeting the only field trial of transgenic trees in the UK, testing trees engineered to suppress lignin production (CAD antisense), was destroyed.

The meeting opened with a keynote address by Ron Sederoff (North Carolina University, USA) in which he succinctly summed up the history of forest biotechnology. He reminded us that in the mid-1980s everyone was saying that they 'didn't believe biotechnology would work in trees'. By the early 1990s people were saying that it 'might work but that it hadn't yet been proven'. And now, in 1999, everyone says they 'knew it was a good idea all along'. Ron went on to further remind us that, despite all the tremendous progress being made, we still face the same fundamental problems, in that the world's forests are still threatened and that we need to learn how to grow better wood. He announced that he and a large group of collaborators were close to final approval for a National Science Foundation pine genome project with a goal of sequencing 50 000 pine xylem ESTs and making these sequences accessible to the public. We have since heard that he and his colleagues were awarded the 3 yr grant. One very exciting element of the project is that this study will focus on wood formation and include components for microarrays and mapping.

Numerous advances were reported in tissue culture, principally in the application of somatic embryogenesis technology. Sara von Arnold from the Swedish University of Agricultural Sciences in Uppsala gave an excellent overview of somatic embryogenesis in conifers and detailed her work with transformation of Norway spruce (*Picea abies*). She also highlighted work with lipid transfer proteins and their regulation in embryo maturation. This presentation was followed by inspiring talks on progress in somatic embryogenesis in *Eucalyptus globulus* (J. Oller, ENCE-DIT, Spain), *Theobroma cacao* (cocoa) (A. Fontanel, Nestlé, France), *Pinus pinaster* (A. Ramarosandratana, Laboratoire des Ressources du Futur, France), *Liquidamber styraciflua* (S. Merkle, University of Georgia, USA) and shoot cultures in *Tectona grandis* (teak) (S. Widiyanto, Institut Teknologi Bandung, Indonesia). Of special interest in these talks was the use of floral tissues for the initiation of embryogenic cultures in *Theobroma* and *Liquidamber* and the fact that in both systems staminate floral parts usually responded better than other floral organs.

The subject of the meeting then switched to transformation with a talk on advances with the use of *Agrobacterium tumefaciens* to transform conifer tissues, in this case white pine, *Pinus strobus* (A. Seguin, Canadian Forest Service, Canada). This work also provided evidence that SARs decreased the variability of gene expression among independently transformed lines. Alternative selection systems for transformed tissue have been a goal for many laboratories and success with the use of the Multi-Auto Transformation Vector system (MAT vectors) was reported (H. Ebinuma, Nippon Paper Industries, Japan). The MAT vectors use an *ipt* gene to induce organogenesis. The *ipt* gene is eventually deleted by the activation of a co-transformed site-specific recombinase acting on excision sites that flank the recombinase and *ipt* genes for removing the gene. Although this work was first reported in 1997 (Proc. Natl. Acad. Sci. USA 94:2117–2121), the talk focused on numerous new extensions of the system such as the use of rolA, B, C and D fragments and the fact that the MAT systems yield a tenfold increase in the recovery of transgenic plants relative to NPT in their hands. Additional reports on transformation included *Ulmus* spp.

*Author to whom correspondence should be addressed; Email elvis@silvagen.com

†http://users.ox.ac.uk/~dops0022/conference/forest_biotech99_home.html

(elm) (K. Gartland, University of Abertay, UK) and an investigation of methylation patterns of rolC inserts in *Populus tremula* and how they may relate to gene silencing (M. Fladung, Institute for Forest Genetics, Germany).

The developmental process of embryogenesis has been investigated by numerous laboratories from a morphological and biochemical viewpoint, yet we still lack good markers to aid us in understanding parameters important for normal embryo maturation and subsequently healthy plants. J. Cairney (Institute of Paper Science and Technology, USA) presented one approach to unravelling the genes involved in the various stages of embryogenesis with the use of a differential display to identify genes expressed at various embryo developmental stages. He reported having a database of over 500 cDNA clones sequenced and the initiation of 'transcript profiling' using DNA microarrays to sort out genes important for different embryo developmental stages. This was followed by other exciting talks detailing work on the cloning and expression of various stress-inducible genes of potential commercial importance. These included an ozone-induced pinosylvin-O-methyltransferase cDNA from *Pinus sylvestris* (D. Ernst, GSF-Institute of Biochemical Plant Pathology, Germany), a polyphenol oxidase induced by wounding, such as insect feeding, from *Populus* spp. (C. P. Constabel, University of Alberta, Canada) and the characterization of an inducible metallothionin gene from *Casuarina glauca* (C. Franche, IRD-GeneTrop, France).

'There is probably no major biochemical process in plants that is both so important and so poorly understood at the molecular level as cellulose synthesis' (Delmer and Amor, Plant Cell 7:987–1000; 1995). While this statement is still true, considerable progress has been made in the past five years in our understanding of this process, as evidenced by the four talks detailing different aspects of cellulose synthesis. An overview of bacterial and plant cellulose synthesis was given by D. Ellis (BC Research Inc., Canada) where the numerous open questions regarding cellulose synthesis were outlined. He presented work from his laboratory on increasing cellulose levels by increasing the level of cellulose precursors and alluded to information that may lead to our ability to modify cellulose properties, such as microfibril size and angle. This was followed by the exciting work on the isolation of a cellulose synthase gene (PtCelA) from aspen and its xylem-specific and stress-inducible expression (C. P. Joshi, Michigan Technological University, USA). The expression of cellulose binding domains (CBD) from cellulase genes was covered by Z. Shani (CBD Technologies, Israel). This group observed increased plant height and cellulose fiber length in plants expressing CBD under the control of the CaMV 35S promoter or a *CelA* promoter. The session finished with a talk on the role of cyclic-di-GMP in plant cellulose synthesis (R. Mayer, Hebrew University of Jerusalem, Israel). The dependence of cyclic-di-GMP for cellulose synthesis in bacterial systems has been known for some time. However, whether it is required for plant cellulose synthesis has previously been unknown. The evidence presented using the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB) certainly suggests a role for cyclic-di-GMP in plant cellulose synthesis.

Emphasis remained on the cell wall but shifted to the large volume of work on lignin modification where more progress has been made in trees than with any other application of genetic engineering. An excellent overview of work done was given by W. Boerjan (University of Gent, Belgium) where he presented data using an OMT promoter–GUS fusion to show how this gene may be involved in the differential deposition of lignin in different tissues. In addition, Boerjan discussed results with the suppression of caffeoyl-CoA-O-methyltransferase where transformed plants contained brown-stemmed phenotype, similar to CAD suppressed plants, in addition to increased pulping efficiency. C. Halpin and co-workers (University of Dundee, UK) are working on methods to coordinately under- and over-express genes. On the over-expression side, they are taking advantage of a viral polyprotein cleavage system to produce polycistronic transcripts of lignin genes which are then processed by viral proteases. Expression in tobacco has given good coordinate regulation. On the gene suppression side, Halpin's group is experimenting with artificial genes containing parts of two or three open reading frames from genes encoding enzymes in the lignin biosynthetic pathway. In addition to demonstrating that these systems work for the coordinated expression of two or more genes, their results with the double antisense inhibition of cinnamyl alcohol dehydrogenase (CAD) with either O-methyltransferase (OMY) or cinnamyl CoA reductase (CCR) resulted in male sterile plants. L. Jouanin (INRA, France) presented the first paper providing evidence that lignin modification may be linked to cellulose synthesis as she found a correlation in plants with decreased OMT and increased cellulose content. She also found that strong antisense suppression (>95%) is required before any phenotypes are observed. J. Grima-Pettenati (Universite Paul Sabatier, France) concluded the information-packed morning session with results from tobacco with decreased CCR levels where an increase in syringyl/guiacyl ratio was observed, as well as the modification of other cell wall components such as phenolics and cellulose.

Perhaps the two most significant talks of the meeting were the back-to-back presentations which provided compelling data that the lignin biosynthetic pathway proposed 30 yr ago is not the preferred pathway in plants and, in fact, may not exist as we were all taught. The pathway had been originally proposed to proceed from phenylalanine through ρ-coumarate to ferulate, where it could either be (1) channelled through coniferaldehyde to coniferyl alcohol to produce guaiacyl residues or (2) through 5-hydroxyferulate through sinapate, sinapaldehyde and sinapyl alcohol to produce syringyl residues. Guaiacyl and syringyl subunits are the two major residues comprising lignin in plants.

C. Chapple (Purdue University, USA) first suggested that syringyl was not produced through ferulate but rather proceeded from ρ-coumarate through ρ-coumaroyl-CoA and caffeoyl-CoA to feruloyl-CoA, the precursor for coniferaldehyde. What was crucial regarding this suggestion was that a gene he previously isolated and characterized, ferulate 5-hydroxylase (F5H), which catalyzed the conversion of ferulate to 5-hydroxyferulate, actually has a 1000-fold lower k_m value for coniferaldehyde and coniferyl alcohol than it has for ferulate. This indicates that the pathway for syringyl lignin in plants likely proceeds through a 5-hydroxylation of coniferaldehyde or coniferyl alcohol, rather than a 5-hydroxylation of ferulate.

V. Chiang (Michigan Technological University, USA) presented some elegant organic and biochemical work which corroborated and complemented the results just reported by Chapple. Again, these results confirmed that the production of both syringyl and guiacyl residues proceeds through caffeoyl-CoA to feruloyl and not through ferulate as was previously proposed. Chiang's observations differed from those of Chapple in that Chiang did not observe the

5-hydroxylation of coniferyl alcohol, although the assay conditions used by the two groups were different. An additional observation noted by Chiang was that the conversion of ferulate to 5-hydroxyferulate was noncompetitively inhibited by coniferaldehyde and the methylation of 5-hydroxyferulate was competitively inhibited by 5-hydroxyconiferaldehyde.

Collectively, these two independent reports very clearly support the notion that the pathway to sinapyl alcohol proceeds not through ferulate, as has been believed for decades, but rather through coniferaldehyde and coniferyl alcohol. Both have since been published (Humphries et al., Proc. Natl. Acad. Sci. USA 96:10045–10050; 1999 and Osakabe et al., Proc. Natl. Acad. Sci. USA 96:8955–8960; 1999). These data also resolve a question that has been lingering for some time, in that 4-coumarate:CoA ligase's (4CL) ability to convert sinapate to sinapoyl-CoA has always been doubtful. In light of these new data, this portion of the pathway does not exist, thereby clarifying why this activity has been difficult to detect. How sinapate esters are formed is now an open question, as F5H is clearly required for the synthesis of these compounds.

C.-J. Tsai (Michigan Technological University, USA) presented work that has since been partially published (Hu et al., Nature Biotechnol. 17:808–812; 1999) on plants with decreased 4CL activity where significant increases were observed in plant growth, including a 7–15% increase in cellulose, height growth, rooting of cuttings and leaf sizes. M. Gray-Mitsumune (University of British Columbia, Canada) discussed the use of antisense to suppress β-glucosidase in spruce to aid our understanding of the role this enzyme plays in lignin production. Although the plants are still young, no significant changes in lignin levels have been observed. J. Dean (University of Georgia, USA; see www.arches.uga.edu/~jeffdean/JDeanUGA.html for pictures of the meeting) discussed his work with a family of laccase genes in yellow poplar. To date, his findings are similar to the β-glucosidase work, in that no phenotype has yet been observed with antisense, whereas ectopic expression resulted in severe abnormalities in the plants. L. Jouanin (INRA, France) demonstrated how arabidopsis could be used for the study of lignin biosynthesis and xylem differentiation with the use of EST databases and a promoterless GUS fusion for xylem-specific promoter trapping. A. Kawaoka (Nippon Paper Industries, Japan) discussed his work on the isolation and characterization of a tobacco PAL-box binding protein, NTLIM1, and presented evidence that antisense suppression of this protein greatly reduces lignin levels by coordinatively down-regulating at least three lignin biosynthetic genes. The final talk of the day was by C. Grunwald (University of Hamburg, Germany) where the examination of wood in 35S-ROLC-transgenic aspen revealed highly abnormal cell wall characteristics and a dwarfing phenotype which is caused by a decrease in the number of cell divisions.

The following day of the meeting was one of contrast as the deployment of transgenic trees was discussed whilst an active protest (complete with drums and someone dressed up in a Frankenstein costume) against the use of genetically engineered trees was going on outside the University Museum. The day began with talks by representatives of The Soil Association (P. Holden and M. Wenban-Smith; www.SoilAssociation.Org), an organization in support of organic farming and completely opposed to the use of GMOs. The concerns they raised included the impact on biodiversity, the lack of containment, unknown and unforeseen consequences of the use of GMOs, and the lack of democratic process in the deployment of the technology. They see the benefits of GMOs as short-lived, nonsustainable and not widespread. They drew a distinction between morally opposed to GMOs and therefore not needing sound scientific rationale for their opposition. For the most part, this was an interesting segment of the meeting as neither party had spoken to the other in an organized nonconfrontational setting previously. Discussions continued for over 2 h the following day between The Soil Association and a small group of the scientists. These discussions did not drastically change the thinking or operations of either party, but they did open up an understanding that all of us involved hope in the future will foster less animosity and greater cooperation to ensure that systems are put in place to study and respond to concerns about the use of genetic engineering in forestry.

S. DiFazio (Oregon State University, USA) followed The Soil Association with results from his studies on the risks of outcrossing of transgenic poplar. This is truly pioneering work in trees and is focused directly at providing experimental tools to assess and look at risk management to limit the spread of transgenic plants into the environment. His studies included measurements on pollen flow, setting up numerous field plots to look at regeneration of non-engineered poplar to assess the rate at which regeneration happens, and data collection to make a model to predict spread by pollen or seed. Results on outcrossing indicate that most outcrossing occurred within 10 m of the parent tree but 74% of the outcrossed pollen came from beyond 1 km so that, clearly, the potential for transgenic pollen spread is high in poplar. S. Maury (IBMP du CNRS, France) then presented work with transgenic tobacco with reduced lignin showing decreased resistance to tobacco mosaic virus, as well as a decreased induction of cell wall phenolics thought to be involved in a plant's defence after wounding, although this later phenomenon is not related to viral resistance.

An overview of several programs, both ongoing and proposed, was presented starting with a UK Forestry program on releasing wild cherry varieties (N. Hammatt, Horticulture Research International, UK). D. Duncan (Monsanto, USA) discussed the proposed joint venture between Monsanto, International Paper, Westvaco and Fletcher Challenge Forests. The joint venture hopes to be a magnet for forestry biotechnology worldwide and would focus on traits such as herbicide resistance, improved growth rates and improved fiber quality. J. Charity (Forest Research, New Zealand) then discussed a program for the transformation of conifers as well as the characterization and isolation of genes involved in reproduction. S. Strauss (Oregon State University, USA) presented an overview of the research being conducted by the Tree Genetic Engineering Co-operative (TGERC) involving transgene flow, Bt and RoundUp Ready field-grown poplars and the control of flowering in poplar.

With the spread of transgenes being a contentious issue, it was no surprise that there were a number of talks investigating reproduction and reproductive structures in trees. O. Nilsson (Swedish University of Agricultural Sciences, Sweden) continued the discussion on flowering with his research investigating the competence to respond to a flowering signal. His work suggested that although a flowering signal such as LFY or AP1 was present, some other factors must be in place for the plant to respond to these homeotic signals and he discussed several arabidopsis mutants which confirmed this hypothesis. This certainly helps explain why numerous researchers have not had success in inducing early

flowering with the ectopic expression of these flowering genes. A. Brunner (Oregon State University, USA) presented very clear data on the expression patterns and characterization of poplar AG and AP1 homologues. J. Skinner (Oregon State University, USA) discussed work using a poplar DEF homologue, PTD, promoter to control a cytotoxin gene in poplar. Co-transforming a 35S-LFY-responding poplar genotype (early flowering) with this cytotoxic construct, they have obtained preliminary evidence that suggests flowering may be disrupted, although the experiments are still in the very early stages. T. Sopanen (University of Joensuu, Finland) presented work on the isolation of arabidopsis floral homologues from birch and the induction of flowers in arabidopsis by expression of a birch AP1 homologue.

R. Rutledge (Canadian Forest Service, Canada) continued the discussion initiated by Nilsson on the competence to respond to a flowering signal. He presented evidence that although a spruce AG homolog and arabidopsis LFY have dramatic effects on flowering when ectopically expressed in arabidopsis, identical constructs in spruce have no effect on the formation of reproductive structures. A. Collins (University of Oxford, UK), described work on phase change in eucalyptus and the isolation of an apparent TFL/CEN ortholog from eucalyptus which complements an arabidopsis TFL mutant. Research involving the evolution of seed plants via analysis of protein and carbohydrates in gymnosperm ovular secretions and their role as barriers to pollination was discussed by P. von Aderkas (University of Victoria, Canada). The final presentation on reproduction dealt with somatic embryogenesis of *Bambusa edulis* and the unusual phenomenon of a proliferating spikelet (spikelet-produce-spikelet) culture in the presence of thidiazuron (W. Chang, Academia Sinica, Taiwan).

Prior to the meeting, IUFRO had begun the process of having each of its working parties draft a position statement on GMOs and plantation forestry. The Working Party on Molecular Genetics was further advanced with this process than other Working Parties, with S. Strauss (Oregon State University, USA) having prepared a draft position statement which was presented at the meeting and was the subject of considerable debate. While many participants felt some statement should be made in response to the public protests at the meeting it was decided by a majority of the participants that the position statement as written could not go forward for one reason or another. The position statement has since been extensively revised and circulated via email to all participants, where it was approved by a majority response. The final position statement can be found at www.fsl.orst.edu/tgerc/iufro pos-statm.htm.

The molecular analysis of tree genomes, although lagging behind that of herbaceous species, is still being carried out by several groups. I. Allona (ETSI Montes Ciudad Universitaria, Spain) presented work on the sequencing of 1097 ESTs from *Pinus taeda* compression wood. This study suggested that there are several unique sequences in pine xylem that are not found in arabidopsis. B. Sundberg (Swedish University of Agricultural Sciences, Sweden) has taken a different approach and asked if arabidopsis could be used to study wood formation. He presented data showing secondary xylem formation in arabidopsis and differential effects on secondary xylem formation with mutations in different ethylene receptor genes. Sundberg ended with a brief introduction to the Swedish poplar EST program where nearly 30 000 ESTs have been sequenced. T. Strabala (Genesis Research and Development Corp. Ltd., New Zealand) discussed what is probably the most ambitious sequencing project in trees to date, the EST sequencing of *Eucalyptus grandis* and *Pinus radiata* with over 100 000 ESTs from each species, making this project comparable to that of *Drosophila*. Numerous important variables involved in managing such a project were discussed, including the fact that despite the large number of sequences already done they are still obtaining novel sequences at a frequency of one in every five. To date, this project has sequenced ESTs from over 30 different libraries including tissue cultured cells.

D. Neale (UDSA-Forest Service, USA) discussed the initiation of an international collaboration, the Conifer Comparative Genomics Project, in which the map of loblolly pine containing nearly 1000 genetic markers is being used as a basis to construct comparative maps for all important pine species. To date, they have found good conservation of gene order on chromosomes among pine species. T. Kondo (Forest Tree Breeding Center, Japan) reported on progress with markers in *Pinus thunbergii* to use in the selection of resistance to pine needle gall midge. A. Seguin (Canadian Forest Service, Canada) discussed recent results on the study of wound-, salicylic acid- and jasmonic acid-induced 14-3-3 protein genes from white spruce and hybrid poplar. C. Plomion (INRA, France) discussed the use of the proteome for protein quantitative locus analysis in *Pinus pinaster* and the generation of a proteomics database. V. Storme (University of Gent, Belgium) discussed the generation of three AFLP maps of *Populus deltoides*, *P. trichocarpa* and *P. nigra* and the alignment of these maps using microsatellites. These data have begun to be used to clone disease-resistant genes in poplar. G. Moran (CSIRO Forest and Forest Products, Australia) has been using RFLP markers and a three-generation pedigree to map QTLs linked to frost tolerance, trunk diameter, height and leaf area in *Eucalyptus nitens*. D. Prat (INRA, France), utilizing AFLP, RAPD and ISSR mapping, has obtained 67 QTL markers with various traits of interest in larch.

The final section of the meeting dealt with the application of molecular genetics and biotechnology in conservation and with novel species. M. Morgante (DuPont, USA) has used a microsatellite approach to map Norway spruce grown in several central European countries, giving ~2100 cM coverage of a 2840 cM genome. Morgante and his collaborators have found a novel transposable element in spruce which they call Alisei. They have developed markers from this element's LTRs and have begun to use it in an AFLP-based approach using an LTR primer and *Eco*RI adapter primers from digested genomic DNA. Five papers (missed by these authors) followed, detailing further genomic characterization through mapping of conifers, oak, wild cherry and the Honduran species *Swietenia humilis*, as well as cryopreservation of *Eucalyptus*.