

# **FOREST BIOTECHNOLOGY '99**

**A Joint Meeting of:**

**The International Wood  
Biotechnology Symposium**

**IUFRO Working Party 2.04-06  
Molecular Genetics of Trees**

**Keble College  
University of Oxford  
Oxford, United Kingdom  
July 11-16, 1999**

# **OFFICIAL PROGRAM**

## **Forest Biotechnology '99**

**A Joint Meeting of:**

**The International Wood Biotechnology Symposium**

**and**

**IUFRO Working Party 2.04-06 Molecular Genetics of Trees**

**Keble College  
University of Oxford  
Oxford, United Kingdom  
July 11-16, 1999**

**Organiser: Malcolm M. Campbell**

**Sponsored by generous donations from:**

**Monsanto Corporation**

**Shell Forestry**

**Oxford Forestry Institute  
and**

**The Department of Plant Science  
University of Oxford**

**This conference would not have been possible without the phenomenal assistance, support, and understanding provided by the following wonderful people:**

**Kim O'Brien  
Liz Pearce  
Jeff Burley  
Janet Betts  
Marilyn Bowler  
Caroline Benfield**

**Angela Collins, Helen Jone, Lusanda Juda, Stephanie McInnis,  
Lisa Newman, Daniel Perazza, Christine Surman, and Janet Willment  
Joan Ouellette  
Callum, Tessa and Connor Campbell**

**SUNDAY, JULY 11, 1999**

- 12:00-20:00 **Registration and Check-In**  
Keble College
- 16:30-18:00 **Welcome**  
University of Oxford Natural History Museum, Lecture Hall  
Jeff Burley, IUFRO President  
Steve Strauss, IUFRO Working Party 2.04-06  
Malcolm Campbell, Conference Organiser
- 17:00-18:00 **Keynote Address**  
University of Oxford Natural History Museum, Lecture Hall
- FOREST BIOTECHNOLOGY:  
WHERE HAS IT BEEN, AND WHERE IS IT GOING?**  
Professor Ronald Sederoff  
North Carolina State University
- 18:00-20:00 **Welcome Mixer**  
University of Oxford Natural History Museum, main hall
- 20:00-23:00 **POSTER VIEWING - Keble College Junior Common Room (JCR)**  
  
all posters should be displayed BUT presenters need not be at posters  
  
cash bar provided

**MONDAY, JULY 12, 1999**

07:30-08:25 Breakfast at Keble College  
08:15 Registration at University of Oxford Natural History Museum

**TISSUE CULTURE AND TRANSFORMATION**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Sarah von Arnold** - Swedish University of Agricultural Sciences, Sweden  
**Marie Connett** - Westvaco, USA

08:30-09:00 Seminar 1: SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE  
S. von Arnold, Swedish University of Agricultural Sciences, Sweden

09:00-09:25 Seminar 2: STUDIES FOR CLONAL PROPAGATION THROUGH  
SOMATIC EMBRYOGENESIS IN EUCALYPTUS GLOBULUS  
J. Oller, ENCE, Spain

09:25-09:45 Seminar 3: IMPROVEMENT OF SOMATIC EMBRYOGENESIS OF  
THEOBROMA CACAO  
A. Fontanel, Centre de Recherche Nestlé Tours, France

09:45-10:10 Seminar 4: IMPORTANCE OF CARBOHYDRATE SOURCE AND PEG  
ON MATURATION OF PINUS PINASTER SOMATIC EMBRYOS:  
YIELD, MORPHOLOGY AND CONVERSION RATE  
A. Ramarosandratana, Laboratoire des Ressources du Futur, France

10:10-10:30 Seminar 5: RAPID IN VITRO PROPAGATION OF ACACIA SPECIES  
K. Nanda, University of Delhi, India

10:30-11:00 **Morning tea at the Natural History Museum**

11:00-11:25 Seminar 6: CLONING OF MATURE LIQUIDAMBAR STYRACIFLUA VIA  
SOMATIC EMBRYOGENESIS FROM DORMANT BUD TISSUES  
S.A. Merkle, University of Georgia, USA

11:25-11:50 Seminar 7: GROWTH RECOVERY AND REGENERATION ABILITY ON  
POST COLD-STORAGE OF SHOOT-TIP CULTURES OF TEAK  
(TECTONA GRANDIS L.)  
S.N. Widiyanto, Institut Teknologi Bandung, Indonesi

11:50-12:15 Seminar 8: STABLE GENETIC TRANSFORMATION OF WHITE PINE  
AFTER COCULTIVATION OF EMBRYOGENIC TISSUES WITH  
AGROBACTERIUM TUMEFACIENS  
A. Séguin, Canadian Forest Service, Canada

12:30 **Lunch at Keble College**

**MONDAY, JULY 12, 1999**

**TISSUE CULTURE AND TRANSFORMATION (continued)**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Sarah von Arnold** - Swedish University of Agricultural Sciences, Sweden  
**Marie Connert** - Westvaco, USA

14:00-14:25 Seminar 9: SELECTION OF MARKER-FREE TRANSGENIC PLANTS USING THE ONCOGENES OF AGROBACTERIUM AS A SELECTABLE MARKER  
H. Ebinuma, Nippon Paper Industries, Japan

14:25-14:50 Seminar 10: TRANSFORMATION, REGENERATION AND GENE EXPRESSION IN ELMS  
K.M.A. Gartland, University of Abertay, Dundee, UK

14:50-15:15 Seminar 11: METHYLATION PATTERN AND FLANKING DNA SEQUENCES IN TRANSGENIC ASPEN-POPULUS LINES IN RELATION TO STABLE OR UNSTABLE TRANSGENE EXPRESSION  
M. Fladung, Institute for Forest Genetics and Forest Tree Breeding, Germany

15:15-15:45 **Coffee break at Natural History Museum**

**MOLECULAR ANALYSIS OF WOODY PLANT TRAITS**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **John Cairney** - Institute of Paper Science and Technology, USA  
**Barry Goldfarb** - North Carolina State University, USA

15:45-16:15 Seminar 12: GENE EXPRESSION DURING LOBLOLLY PINE EMBRYO DEVELOPMENT: TRANSCRIPT PROFILING AND GENE REGULATION STUDIES  
J. Cairney, Forest Biology Group, Institute of Paper Science and Technology, USA

16:15-16:45 Seminar 13: ABA SIGNAL TRANSDUCTION DURING INDUCTION OF BUD DORMANCY IN POPLAR  
A. Rohde, University of Gent, Belgium

16:45-17:10 Seminar 14: MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF A STRESS-INDUCED PINOSYLVIN-O-METHYLTRANSFERASE cDNA FROM SCOTS PINE (PINUS SYLVESTRIS L.)  
D. Ernst, GSF-Institute of Biochemical Plant Pathology, Germany

17:10-17:20 **Break**

17:20-17:45 Seminar 15: MOLECULAR APPROACHES TO PEST DEFENSE: CLONING AND CHARACTERIZATION OF HYBRID POPLAR AND TREMBLING ASPEN POLYPHENOL OXIDASE  
C.P. Constabel, University of Alberta, Canada

17:45-18:05 Seminar 16: CHARACTERIZATION OF A METALLOTHIONEIN GENE FROM THE ACTINORHIZAL TREE CASUARINA GLAUCA  
C. Franche, IRD-GeneTrop, France

**MONDAY, JULY 12, 1999**

18:05-18:35 Seminar 17: AUXIN-INDUCED GENES FROM LOBLOLLY PINE AND  
THEIR ROLE IN DEVELOPMENT  
B. Goldfarb, North Carolina State University, USA

19:00 **Dinner at Keble College**

20:00-23:00 **POSTER SESSION - Keble College Junior Common Room (JCR)**

all posters should be displayed

presenters with **ODD NUMBERED POSTERS** should be at their  
posters

cash bar provided

## TUESDAY, JULY 13, 1999

07:30-08:25 Breakfast at Keble College  
08:15 Registration at University of Oxford Natural History Museum

### THE MOLECULAR BIOLOGY AND DIRECTED MODIFICATION OF WOOD (Session 1)

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Wout Boerjan**- University of Gent, Belgium  
**David Ellis** - BC Research, Canada

08:30-09:00 Seminar 18: CELLULOSE – COVERING YOUR EMBARRASSING PARTS AND OTHER STORIES  
D. Ellis, BC Research, Canada

09:00-09:25 Seminar 19: A NOVEL XYLEM-SPECIFIC AND TENSION STRESS RESPONSIVE CELLULOSE SYNTHASE (PtCelA) GENE FROM QUAKING ASPEN  
C.P. Joshi, Michigan Technological University, USA

09:25-09:50 Seminar 20: INCREASING POLYSACCHARIDE BIOSYNTHESIS VIA EXPRESSION OF CELLULOSE BINDING DOMAIN (CBD) IN TRANSGENIC PLANTS.  
Z. Shani, CBD Technologies, Ltd., Israel

09:50-10:15 Seminar 21: REGULATION OF PLANT CELLULOSE BIOSYNTHESIS BY C-DI-GMP  
R. Mayer, The Hebrew University of Jerusalem, Israel

10:15-10:45 **Morning tea at the Natural History Museum**

10:45-11:15 Seminar 22: UNRAVELING LIGNIN BIOSYNTHESIS IN POPLAR  
W. Boerjan, University of Gent, Belgium

11:15-11:40 Seminar 23: ENABLING TECHNOLOGIES FOR THE COMPLEX MANIPULATION OF LIGNIN BIOSYNTHESIS  
C. Halpin, University of Dundee, UK

11:40-12:05 Seminar 24: NEW INSIGHTS ON LIGNIN ENGINEERING OF POPLARS: IMPACTS OF EXTREMELY LOW CAD AND OMT ACTIVITIES  
L. Jouanin, INRA Versailles, France

12:05-12:30 Seminar 25: CINNAMOYL CoA REDUCTASE, THE FIRST COMMITTED STEP OF THE LIGNIN BRANCH PATHWAY: EXPRESSION AND DOWN-REGULATION THROUGH ANTISENSE STRATEGY  
J. Grima-Pettenati, UMR CNRS/ Université Paul Sabatier, France

12:30 **Lunch at Keble College**

**TUESDAY, JULY 13, 1999**

**THE MOLECULAR BIOLOGY AND DIRECTED MODIFICATION OF WOOD  
(Session 2)**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Brian Ellis** - University of British Columbia, Canada  
**Clint Chapple**- Purdue University, USA

14:00-14:30 Seminar 26: PHENYLPROPANOID METABOLISM IN ARABIDOPSIS:  
BIOCHEMISTRY AND BIOTECHNOLOGY  
C. Chapple , Purdue University, USA

14:30-14:55 Seminar 27: A CLARIFICATION OF SYRINGYL MONOLIGNOL  
BIOSYNTHESIS  
L. Li, Michigan Technological University, USA

14:55-15: 20 Seminar 28: ENHANCED GROWTH AND CELLULOSE  
ACCUMULATION IN LIGNIN-REDUCED TRANSGENIC ASPEN WITH  
DOWN-REGULATED 4-COUMARATE:CoA LIGASE  
C.-J. Tsai, Michigan Technological University, USA

15:20-15:45 **Coffee break at Natural History Museum**

15:45-16:15 Seminar 29: GENETIC MODIFICATION OF THE LIGNIN  
BIOSYNTHETIC PATHWAY IN SPRUCE VIA DOWN-REGULATION  
OF THE CONIFERIN BETA-GLUCOSIDASE GENE  
M. Gray-Mitsumune, University of British Columbia, Canada

16:15-16:40 Seminar 30: CHARACTERIZATION AND EXPRESSION OF LACCASE  
GENES FROM YELLOW-POPLAR XYLEM  
J.F.D. Dean, University of Georgia, USA

16:40-17:00 Seminar 31: ARABIDOPSIS THALIANA: A MODEL PLANT FOR  
LIGNIFICATION STUDIES  
L. Jouanin, INRA Versailles, France

17:00-17:25 Seminar 32: TOBACCO TRANSCRIPTION FACTOR NTLIM1  
REGULATES LIGNIN BIOSYNTHESIS  
A. Kawaoka, Nippon Paper Industries, Japan

17:25-17:50 Seminar 33: WOOD STRUCTURE AND CELL WALL ARCHITECTURE  
IN RoIC TRANSGENIC ASPEN TREES  
C. Grünwald, University of Hamburg, Germany

19:00 **Dinner at Keble College**

20:00-23:00 **POSTER SESSION - Keble College Junior Common Room (JCR)**

all posters should be displayed

presenters with **EVEN NUMBERED POSTERS** should be at their  
posters

cash bar provided



**WEDNESDAY, JULY 14, 1999**

07:30-08:25 Breakfast at Keble College  
08:15 Registration at University of Oxford Natural History Museum

**FROM LAB TO FIELD - THE APPLICATION OF FOREST TREE MOLECULAR BIOLOGY AND WOOD BIOTECHNOLOGY IN FORESTRY**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **John Purse** - Shell Forestry, UK  
**Steve Strauss** - Oregon State University, USA

08:30-09:00 Seminar 34: THE CASE AGAINST GENETIC ENGINEERING IN FARMING AND FORESTRY  
P. Holden & M. Wenban-Smith, The Soil Association, UK

09:00-09:25 Seminar 35: OUTCROSSING RISKS FOR TRANSGENIC HYBRID POPLARS  
S. DiFazio, Oregon State University, USA

09:25-09:50 Seminar 36: TRANSGENIC TOBACCOS WITH DEPRESSED OMTs HAVE REDUCED LIGNIN CONTENT, MODIFIED VASCULAR ORGANISATION AND WEAKENED ANTI-VIRAL RESISTANCE  
S. Maury, IBMP du CNRS, France

09:50-10:15 Seminar 37: RELEASING WILD CHERRY CLONES TO THE UK FORESTRY INDUSTRY- THE CHALLENGE  
N. Hammatt, Horticulture Research International, UK

10:15-10:30 **Morning tea at the Natural History Museum**

10:30-11:00 Seminar 38: FORESTRY BIOTECHNOLOGY JOINT VENTURE  
David Duncan, Monsanto, USA

11:00-11:25 Seminar 39: GENETICALLY ENGINEERED TREES FOR COMMERCIAL FORESTRY  
J.A Charity, Forest Research, New Zealand

11:25-11:50 Seminar 40: GENE FLOW FROM TRANSGENIC PLANTATIONS: PREVENTION AND ANALYSIS  
S. Strauss, Oregon State University, USA

11:50-12:30 DISCUSSION OF POSITION PAPER:  
"GENETIC ENGINEERING OF FOREST TREES"  
S. Strauss, Oregon State University, USA

12:30 **Lunch at Keble College**

**WEDNESDAY, JULY 14, 1999**

**THE MOLECULAR BIOLOGY OF FLOWERING IN TREES**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Ove Nilsson** - Swedish University of Agricultural Sciences, Sweden  
**Robert Rutledge** - Canadian Forest Service, Canada

- 14:00-14:30 Seminar 41: REGULATION OF FLOWERING TIME IN TREES  
O. Nilsson, Swedish University of Agricultural Sciences, Sweden
- 14:30-14:55 Seminar 42: GENES CONTROLLING FLOWERING IN POPULUS:  
HOMOLOGS OF THE ARABIDOPSIS GENES APETALA1 (AP1) AND  
AGAMOUS (AG)  
A. Brunner, Oregon State University, USA
- 14:55-15:20 Seminar 43: ENGINEERING OF CYTOTOXIN-BASED GENETIC  
STERILITY IN POPLAR USING THE PROMOTER OF A DEFICIENS-  
LIKE GENE FROM POPULUS TRICHOCARPA  
J.S. Skinner, Oregon State University, USA
- 15:20-15:45 Seminar 44: TOWARDS NON-FLOWERING BIRCHES  
J. Lemmetyinen, University of Joensuu, Finland
- 15:45-16:15 **Coffee break at Natural History Museum**
- 16:15-16:40 Seminar 45: GENETIC REGULATION OF FLOWERING: EXPLORING  
POTENTIAL APPLICATIONS TO MANIPULATING CONE FORMATION  
IN CONIFERS  
R.G. Rutledge, Canadian Forest Service, Canada
- 16:40-17:00 Seminar 46: MOLECULAR DISSECTION OF PHASE CHANGE IN  
EUCALYPTUS  
A.J. Collins, University of Oxford, UK
- 17:00-17:25 Seminar 47: OVULAR SECRETIONS IN SEED PLANTS: EVOLUTION  
AND BIOCHEMICAL COMPLEXITY  
Patrick von Aderkas, University of Victoria, Canada
- 17:25-17:50 Seminar 48: FLOWERING AND SPIKELET PROLIFERATION OF  
BAMBUSA EDULIS IN VITRO  
W.C. Chang, Academia Sinica, Taiwan
- 19:00 **Dinner at Keble College**
- 20:30 **Business meeting**  
Keble College Dining Hall
- Choice of location for next meeting**  
**Continuation of discussion of position paper on "Genetic**  
**Engineering of Forest Trees"**  
Cash bar provided in Keble College JCR

**THURSDAY, JULY 15, 1999**

07:30-08:25 Breakfast at Keble College  
08:15 Registration at University of Oxford Natural History Museum

**MOLECULAR ANALYSIS OF TREE GENOMES AND TREE GENETICS  
(Session 1)**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **Isabel Allona** - ETSI Montes, Spain  
**Christophe Plomion**- INRA, France

08:30-09:00 Seminar 49: THE USE OF RANDOM SEQUENCING IN THE ANALYSIS OF XYLEM CELL WALL FORMATION IN PINE  
Isabel Allona, ETSI Montes, Spain

09:00-09:30 Seminar 50: POPLAR AND ARABIDOPSIS AS MODEL SYSTEMS FOR WOOD FORMATION  
Björn Sundberg, SLU, Sweden

09:30-10:00 Seminar 51: THE GENESIS PINE AND EUCALYPTUS EST PROJECTS.  
Timothy J. Strabala, Genesis Research & Development Corp., New Zealand

10:00-10:30 **Morning tea at the Natural History Museum**

10:30-11:00 Seminar 52: CONIFER COMPARATIVE GENOMICS PROJECT  
David B. Neale, Institute of Forest Genetics, USA

11:00-11:25 Seminar 53: RAPD MARKERS LINKED TO A GENE FOR RESISTANCE TO PINE NEEDLE GALL MIDGE IN JAPANESE BLACK PINE (PINUS THUNBERGII)  
T. Kondo, Forest Tree Breeding Center, Japan

11:25-11:50 Seminar 54: GENES POTENTIALLY INVOLVED IN THE SIGNAL TRANSDUCTION PATHWAY LEADING TO TREE DEFENSE AGAINST PATHOGENS: 14-3-3 CDNA CLONES ISOLATED IN WHITE SPRUCE AND HYBRID POPLAR  
A. Seguin, Canadian Forest Service, Canada

11:50-12:15 Seminar 55: QUANTITATIVE TRAIT LOCI (QTLS) FOR ECONOMICALLY IMPORTANT TRAITS IN PINUS SYLVESTRIS  
E. Lerceteau, SLU, Sweden

12:30 **Lunch at Keble College**

THURSDAY, JULY 15, 1999

**MOLECULAR ANALYSIS OF TREE GENOMES AND TREE GENETICS  
(Session 2)**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:**        **David Neale** - Institute of Forest Genetics, USA  
                  **Timothy Strabala** - Genesis Research and Development Corp., New Zealand

14:00-14:30    Seminar 56: GENETICS OF THE MARITIME PINE PROTEOME  
                  C. Plomion, INRA, France

14:30-15:00    Seminar 57: GENETIC MAPPING OF POPLAR  
                  V. Storme, University of Gent, Belgium

15:00-15:25    Seminar 58: LINKING PHYSIOLOGICAL TRAITS TO MOLECULAR  
                  MARKERS: PUTATIVE QTL FOR LEAF GROWTH IN POPLAR  
                  G. Taylor, University of Southampton, UK

15:25-15:55    Seminar 59: QTL FOR GROWTH IN EUCALYPTUS NITENS  
                  G.F. Moran, CSIRO Forestry and Forest Products, Australia

15:55-16:15    Seminar 60: QTL DETECTION IN A FACTORIAL MATING DESIGN:  
                  ANALYSIS OF WOOD MICRODENSITOMETRY CHARACTERS IN  
                  LARCH  
                  D. Prat, INRA Centre d'Orleans, France

16:15            **Free Time**

18:00            Cash bar available at Keble College

20:00            **Conference Banquet - Keble College Dining Hall**

**FRIDAY, JULY 16, 1999**

07:30-08:25 Breakfast at Keble College  
08:15 Registration at University of Oxford Natural History Museum

**THE APPLICATION OF MOLECULAR GENETICS AND BIOTECHNOLOGY IN CONSERVATION AND WITH NOVEL SPECIES**

University of Oxford Natural History Museum, Lecture Hall

**Chairs:** **David Boshier** - University of Oxford, UK  
**Michele Morgante** - EI DuPont de Nemours and Co., USA

08:30-09:00 Seminar 61: BIOTECHNOLOGY, GENOMICS AND BIODIVERSITY: HOW CAN WE PUT THEM ALL TOGETHER IN NORWAY SPRUCE?  
M. Morgante, EI DuPont de Nemours and Co.

09:00-09:25 Seminar 62: DISTRIBUTION OF THE HAPLOTYPIC DIVERSITY IN CONIFER AND BROADLEAVED SPECIES AS REVEALED BY CHLOROPLAST MICROSATELLITES  
G.G. Vendramin, Istituto Miglioramento Genetico Piante Forestali, Italy

09:25-09:50 Seminar 63: MICROSATELLITE ANALYSIS OF SEEDLOT SAMPLES FROM PEDUNCULATE OAK: INFERENCE OF THE SEED PARENTS AND POLLEN DONORS FROM THE OFFSPRING  
C. Lexer, Agricultural University of Vienna, Austria

09:50-10:15 Seminar 64: MOLECULAR GENETIC INVESTIGATIONS IN WILD BIRD CHERRY AND SWEET CHERRY CULTIVARS (PRUNUS AVIUM L.) IN CENTRAL EUROPE  
B. Heinze, Federal Forest Research Centre, Austria

10:15-10:30 **Morning tea at the Natural History Museum**

10:30-11:00 Seminar 65: THE DYNAMICS OF MATING IN A FRAGMENTED POPULATION OF SWIETENIA HUMILIS (ZUCC.) USING SSRS AS A MARKER SYSTEM  
D. Boshier, University of Oxford, UK

11:00-11:25 Seminar 66: RAPD DETECTION OF NARROW-LEAF PHENOTYPES IN THE REGENERATED PLANTLETS OF HYBRID POPLAR  
K. Ishii, Forestry and Forest Products Research Institute, Japan

11:25-11:50 Seminar 67: APPLICATION OF CRYOPRESERVATION TO A CLONAL EUCALYPTUS IMPROVEMENT PROGRAMME  
D Blakesley & R Kiernan, Horticulture Research International, UK

11:50-12:20 Seminar 68: BIOTECHNOLOGY OF POPULUS EUPHRATICA IN INDIAN SEMI-ARID TRANS GANGETIC PLAINS  
A. Sharma, Tata Energy Research Institute, India.

12:20-12:30 **Farewell to Conference Participants**  
**Malcolm Campbell**

**End Of Scientific Programme for Forest Biotechnology '99**

**SUNDAY, JULY 11, 1999**

**KEYNOTE ADDRESS**

University of Oxford Natural History Museum,  
Lecture Hall

## **FOREST BIOTECHNOLOGY, WHERE HAS IT BEEN, AND WHERE IS IT GOING?**

Ron Sederoff

Departments of Forestry, Genetics and Biochemistry, North Carolina State University, USA

volvo@unity.ncsu.edu

Although there are many landmarks in the brief history of forest biotechnology, one starting point is the creation of the first transgenic tree in 1987. Within the short period of time that has followed, we have seen the rapid development of genetic mapping in trees, and the incorporation of mapping technology into many tree-breeding programs. Genomic mapping was the first phase of what has now become tree genomics, which extends mapping to the level of DNA sequence. Transgenic technology for forest trees has moved into intense commercial application and is now becoming integrated with genomics and high throughput gene discovery. The future clearly holds promise for directed modification of many characteristics of forest trees, particularly related to wood properties, growth and development and adaptation to pests, pathogens and environmental stresses. The power of the approach follows from the prospects of understanding of the functions of virtually all the genes. This daunting task will require the integrated study of model plant systems as well as the trees themselves. It is reasonable to expect that genomics of forest trees will lead to the identification of most of the genes in some tree species, and that such genes will be mapped, and some features of function will be known for most of the genes. The major remaining task, even more daunting, is the association of genetic variation with phenotypes of commercial or biological importance through quantitative analysis of pedigrees and natural populations. Genomic approaches will open the door to understanding the extent and nature of epistasis in domesticated and undomesticated trees. A major challenge is to use the power of biotechnology to aid in conservation of forest species, and to improve the efficiency of plantation forestry. Genome level understanding of dominant species in specific ecosystems will make possible molecular approaches to landscape ecology. These needs are particularly important, in the face of increasing demands on the land, and the possibility of biotechnology based ecoterrorism. An update on the loblolly pine genome project will also be included.

**MONDAY, JULY 12, 1999**

**TISSUE CULTURE AND TRANSFORMATION**

University of Oxford Natural History Museum,  
Lecture Hall



## **SEMINAR 1**

### **SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE**

S. von Arnold, P. Bozhkov, D. Clapham and I. Sabala

Forest Genetics, SLU (Swedish University of Agricultural Sciences), Sweden

Sara.von.Arnold@sgen.slu.se

<http://www.sgen.slu.se/Skogsgenetik/staffmapp/sara.home.html>

Plant regeneration via somatic embryogenesis has practical application in forestry. In addition the process offers unique opportunities to study embryology. The method to regenerate plants from somatic embryos of Norway spruce has been improved, especially the maturation step. At least one third, out of 600 tested genotypes, can be propagated via somatic embryos when using a standard protocol. The possibilities to integrate somatic embryos in a breeding programme of Norway spruce is now tested. A particle inflow gun enables effective transfer of genes to embryogenic cultures of Norway spruce and transgenic plants are regenerated. In our attempts to understand the regulation of somatic embryo development in Norway spruce we are searching for markers for specific stages, regulatory genes and signal molecules. A full length cDNA clone Pa18, encoding a protein with the characteristics of plant lipid transfer proteins, has been isolated and characterized. The Pa18 gene is specifically expressed in the "protodermis" in maturing somatic embryos. In transgenic cell lines over- and under expressing Pa18, normal embryo development is disturbed. Regulatory genes, belonging to the HD-GL2 family as well as homeobox genes, are expressed in somatic embryos. Extracellular signal molecules regulate early stages of embryo development.

## SEMINAR 2

### STUDIES FOR CLONAL PROPAGATION THROUGH SOMATIC EMBRYOGENESIS IN EUCALYPTUS GLOBULUS

J. Oller

ENCE - DIT, Spain

oller@ence.es

[http://www.ence.es/uk/el\\_grupo.htm](http://www.ence.es/uk/el_grupo.htm)

Somatic embryogenesis applied to a commercially interesting species such as *Eucalyptus globulus* is an efficient procedure to cultivate tissues. This system produces high clonal multiplication of pre-selected trees from artificial seeds, as well as providing an optimum mechanism for gene transfer and for the production of transgenic trees. The results we have achieved have enabled us to reach the embryogenic calli induction phase, by using leaf tissue cultivated in a BMO basal medium supplemented by a 2,4-D/NAA (0.5-5.0 µM) medium followed by a medium with a low macronutrient content.

These studies are financed by the European Union (FAIR project nº CT95-0424).

### **SEMINAR 3**

#### **IMPROVEMENT OF SOMATIC EMBRYOGENESIS OF THEOBROMA CACAO L.**

A. Fontanel, M. Alvarez, O. Lopez-Baez, G. Labbé, and V. Pétiard

Centre de Recherche Nestlé Tours, France (AF,GL,VP), Nestlé RandD Center Quito (MA) and INIFAP CE Rosario Izapa (OLB)

annie.fontanel@rdto.nestle.com

A successful procedure for cacao regeneration has been developed by inducing somatic embryogenesis of flower parts. The first plants derived from primary somatic embryos are currently being field tested in Ecuador. Almost all of the 200 plants showed growth characteristics that were similar to seed-derived plants. The plants were producing pods, however, a proper assessment of productivity was not yet possible due to the unusual climatic conditions of 1998. The application of somatic embryogenesis on a larger scale will require further optimisation. One of the key steps towards this goal is the improvement of the multiplication rate by the development of secondary embryogenesis in solid or liquid media. The results of these studies will be discussed more in details during this presentation.

## SEMINAR 4

### IMPORTANCE OF CARBOHYDRATE SOURCE AND PEG ON MATURATION OF PINUS PINASTER SOMATIC EMBRYOS: YIELD, MORPHOLOGY AND CONVERSION RATE

A. Ramarosandratana, L. Harvengt, E. Garin, F. Canlet, M. Pâques, and R. Calvayrac

Laboratoire des Ressources du Futur, Nangis, France (AR, LH, EG, FC and MP) and  
Laboratoire des Membranes Biologiques, Université Paris, France (RC)

biotech@afocel.fr

[http://www.afocel.fr/index\\_e.html](http://www.afocel.fr/index_e.html)

Maturation of somatic embryos has been investigated in maritime pine (*Pinus pinaster*). Previous studies showed that well-developed mature embryos were obtained independently the nature of the carbon source (maltose or sucrose) when high concentration of gellan gum was used. Moreover, the use of PEG greatly enhanced the production of mature embryos (2-4 times). Increasing sucrose concentration (9%, w/v) decreased the yield of maturation, whereas the number of embryos progressively increased with maltose concentration. Despite the maturation was favoured by high gellan gum concentration, numerous well-formed embryos (500 per g of FW) were developed with low gel strength medium when maltose is the carbon source. This result indicates that maturation of maritime pine somatic embryos could be obtained on media with a weaker gel strength, and even on liquid medium when using maltose as carbon source. Morphometric analyses showed that morphology and length of embryos are influenced by the type and the concentration of sugar. Furthermore, the addition of PEG4000 at 6% significantly increased the embryo length (10-20%) depending on the sugar. No correlation between the embryo length and the ability to germinate was observed. Desiccation improved the survival of PEG treated embryos but derived plantlets failed in developing roots. Plantlets from these treatments were successfully acclimatized and their growth traits are steadily compared.

## SEMINAR 5

### RAPID IN VITRO PROPAGATION OF ACACIA SPECIES

K. Nanda, S.Philips, and A. Dewan

Department of Botany, Daulat Ram College, University of Delhi, India (KN, SP) and AND College (AD)

khatris@ndb.vsnl.net.in

Realising the immense economic and ecological values and dwindling population of Acacias - the saviour trees of arid zones, mass micropropagation protocols have been developed for *Acacia holosericea*, *A. senegal* and *A. tortilis*. Juvenile explants viz. Cotyledons (COT) cotyledonary nodes (CN), leaflets and leaf rachis excised from 15-day-old seedlings of *A. HOLOSERICEA* showed significant morphogenic response when cultured on Gamborg et al's B5 medium adjuvanted with various cytokinins. Within 15-20 days of culture on 2.5mg/l zeatin supplemented B5 medium, the COT and leaflets hypertrophied. Multiple shoot buds differentiated either directly or via callus. Forty per cent of the COT cultures developed an average of  $4.3\pm 2.7$  shoots per explant, while 16% of the leaflets had organised an average of  $3.0\pm 1.0$  shoots per explant. The caulogenic response was enhanced in COT to 50% with an average of  $12.0\pm 6.8$  shoots per explant and that of leaflets to 33% and  $7.0\pm 3.8$  shoots per explant by addition of 200 mg/l adenine sulphate to the medium. In *A. tortilis* maximum shoot production (95%) with an average of  $3.07\pm 0.9$  shoots per explant was obtained from CN cultured on Modified MS with 2 mg/l BA. An average of 4.2 shoots per explant was observed in cent percent CN of *A. senegal* when cultured on MS containing 3.5 mg/l BA. Shoots of *A. holosericea* differentiated direct roots in 100% explants on B5 basal medium only. While for *A. tortilis* 1/2 strength MS with 2 mg/l IBA proved optimum and cent per cent shoots developed long healthy roots. *In vitro* regenerated plantlets have been successfully transferred to soil.

## SEMINAR 6

### CLONING OF MATURE LIQUIDAMBAR STYRACIFLUA VIA SOMATIC EMBRYOGENESIS FROM DORMANT BUD TISSUES

S.A. Merkle and P.J. Battle

Daniel B. Warnell School of Forest Resources, University of Georgia, USA

smerkle@arches.uga.edu

<http://www.forestry.uga.edu/wsfr1/faculty/html/merkle.html>

Floral and inflorescence tissues have proven to be useful explants for embryogenic culture initiation for a number of woody perennials via indirect somatic embryogenesis. The ability to use such tissues, rather than those of genetically unproven seeds or seedlings, offers an important advantage for rapid improvement of forest trees, since it makes possible mass cloning of trees that are sufficiently mature to be evaluated for superior growth and other qualities. Staminate inflorescences, pistillate inflorescences and leaves collected from dormant buds of three sweetgum (*Liquidambar styraciflua*) trees were tested for induction of somatic embryogenesis following treatment with thidiazuron, naphthaleneacetic acid (NAA) or different combinations of the two. Explants were placed into culture either within a few days after collection or following two months of storage at -15 C. Embryogenesis induction was strongly affected by source tree and explant type. Staminate inflorescences were up to five times more likely to produce an embryogenic culture as female inflorescences and no embryogenic cultures were obtained from leaf explants. While culture on medium with NAA alone resulted in the highest production of repetitively embryogenic cultures and cultures producing proembryogenic masses, some embryogenic cultures were initiated from inflorescences cultured on medium with no plant growth regulators at all. Dormant buds stored for two months at -15 C were still capable of producing embryogenic cultures, although frozen storage decreased this ability by over one-half for staminate inflorescences.

## SEMINAR 7

### **GROWTH RECOVERY AND REGENERATION ABILITY ON POST COLD-STORAGE OF SHOOT-TIP CULTURES OF TEAK (*TECTONA GRANDIS* L.)**

S.N. Widiyanto, S. Sisunandar, and E. Riani

Department of Biology, Institut Teknologi Bandung, Indonesia

srinanan@bi.itb.ac.id

Shoot-tips of teak (*Tectona grandis* L.), collected from field, were potential explants for clonal propagation. Shoot-tips were cultured on shoot induction media contained modified Murashige and Skoog (MS) medium. The addition of 0.5 to 5.0 micromolar 6-benzyladenine (BA) and 0.5 to 5.0 micromolar alpha-naphthaleneacetic acid (NAA) induced shoot growth. Shoot proliferation was established on medium consisted of woody-plant-medium supplemented with 2.0 to 8.0 micromolar BA and 0.01 to 0.1 micromolar 2,3,5-triiodobenzoic acid (TIBA). The average of 4.0 shoots were produced per explant-shoot in four weeks. Shoot-tips, excised from sterile shoots, were subjected to a cold-storage condition. Dimethylsulfoxide (DMSO) was used as a cryoprotectant prior to fast-freezing process. Cryoprotective treatment was carried out on liquid MS medium added with various concentrations of DMSO at 5 to 15 percent (volume per volume). Pretreated shoot-tips were directly frozen in sterile condition at minus 4 and 0 degree Celsius. After a period of cold-storage (1 to 10 days), shoot-tips were thawed and precultured on shoot recovery medium. The results showed that without DMSO treatment, frequency of shoot-tip recovery was low. The use of 5 percent DMSO as cryoprotectant was more effective than that of the 10 percent and 15 percent. Fifty percent of shoot-tips remained viable after a three-day period of cold-storage at 0 degree Celsius. With the 5 percent DMSO treatment, shoot-tips showed the capability to recover and regrow after a period of five-day cold-storage. Recovered teak shoots retained their regrowth and regenerated into plantlets.

## SEMINAR 8

### STABLE GENETIC TRANSFORMATION OF WHITE PINE AFTER COCULTIVATION OF EMBRYOGENIC TISSUES WITH AGROBACTERIUM TUMEFACIENS

V. Levée, E. Garin, K. Klimaszewska and A. Séguin

Natural Resources Canada, Canadian Forest Service, Canada

seguin@cfl.forestry.ca  
<http://www.cfl.forestry.ca>

A genetic transformation procedure for white pine has been developed after cocultivation of embryogenic tissues with *Agrobacterium tumefaciens*. This efficient transformation procedure led to an average of four independent transformed lines per gram of cocultivated embryogenic tissue and up to 50 transformed lines can be obtained in a routine experiment. Constructs bearing the uidA gene or the green fluorescent protein (GFP) gene were introduced and beta-glucuronidase (GUS) activity was followed over time. The expression of the uidA gene was lowest with a 35S-gus-intron construct and was 20-fold higher with a 35S-35S-AMVgus::nptII construct. The addition of scaffold attachment region (SAR) sequences surrounding the gus::nptII fusion did not significantly enhance the GUS activity. Transformed mature somatic embryos have been germinated and plantlets have been acclimatized.



## SEMINAR 9

### SELECTION OF MARKER-FREE TRANSGENIC PLANTS USING THE ONCOGENES OF AGROBACTERIUM AS A SELECTABLE MARKER

H. Ebinuma, K. Sugita, E. Matsunaga, S. Endo, and T. Kasahara

Central Research Laboratory, Nippon Paper Industries, Japan

LDW06374@nifty.ne.jp

[http://www.npaper.co.jp/e\\_html/index.html](http://www.npaper.co.jp/e_html/index.html)

*Agrobacterium* has been widely used to produce transgenic plants. We have utilized only *Agrobacterium*-mediated gene transfer system and have not used plant hormone regulation system of oncogenes for transformation. Alternatively, we have applied exogenous plant hormones to regenerate and proliferate transgenic tissues. Oncogenes appear to have high flexibility for the regulation of endogenous plant hormonal levels. However, oncogenes have not been used to induce proliferation and differentiation of transgenic cells because the regenerated transgenic plants exhibit a seriously abnormal phenotypes. The Multi-Auto-Transformation Vector System is designed to regenerate transgenic plants by using the oncogenes and remove them to recover the phenotypic normality of transgenic plants by using yeast site-specific recombination system R/RS (Ebinuma *et al.*, 1997a,b, Sugita *et al.*, in press). In the MAT vectors, the chimeric ipt gene or the rol genes combined with the recombinase (R) gene were placed within two directly oriented recognition sites (RS) to remove them from transgenic cells after the transformation. When we used the MAT vectors for transformation of tobacco and hybrid aspen, the transgenic cells containing the ipt gene regenerated into the ipt-shooty phenotype on the hormone-free medium. During cultivation of the ipt-shooty lines, we could obtain marker-free transgenic plants as normal plants that recovered apical dominance. To increase the number of transgenic plants and marker-free transgenic plants, we fused the ipt and R genes with different promoters and inserted into the MAT cassette to create new MAT vectors. We introduce improved version of MAT vectors for practical uses and their efficient application in woody plants.

## SEMINAR 10

### TRANSFORMATION, REGENERATION AND GENE EXPRESSION IN ELMS

K.M.A. Gartland, J.S. Gartland, A.T. McHugh, T.M. Fenning, R.J. Irvine and C.M. Brasier

Molecular and Life Sciences, University of Abertay, Dundee, UK (KMAG, JSG, ATMCh, RJI), Forest Research, Alice Holt, Surrey, UK (CMB), Max Planck Inst. Chemical Ecology, Jena, Germany (TMF)

k.gartland@tay.ac.uk  
<http://hirta.tay.ac.uk>

Elms (*Ulmus* spp.) are highly prized for their landscape and timber qualities. Millions of elms have been lost to the Dutch elm disease pathogen *Ophiostoma novo-ulmi*. Genetic modifications may overcome the lack of success achieved by traditional breeding using European elms allowing the introduction of desirable disease tolerance associated genes into elite genotypes. Micropropagation and regeneration systems have been established for English elm (*U. procera*), wych elm (*U. glabra*) and the American elm (*U. americana*). Genetic modifications have been carried out using *Agrobacterium* species. Transformed *U. procera* regenerants have been obtained using wild-type *A. tumefaciens* strains, as confirmed by DNA-DNA hybridisations and nopaline assays. These plants were of abnormal morphology, due to T-DNA oncogene expression. Transformed regenerants have also been produced using the *A. Rhizogenes* Ri plasmid, as confirmed by PCR and DNA sequencing. Regenerants exhibited varying degrees of dwarfing, reduced apical dominance and epinasty, due to Ri plasmid T-DNA gene expression. Modifications to elm anatomy may be of value in investigating the DED infection process. Variations in GUS reporter gene expression has been shown in leaves, stems and roots for series of disarmed *A. tumefaciens* genetically modified regenerants. Developmental assessments of transgene expression have been carried out. Genes associated with potential pathogen tolerance have been introduced into elms. Their effects on elm growth, development and disease susceptibility will be investigated. These data will provide valuable indicators for the control of transgene expression in broadleaf trees and provide a biotechnological approach to fighting the ravages of Dutch elm disease.

## SEMINAR 11

### METHYLATION PATTERN AND FLANKING DNA SEQUENCES IN TRANSGENIC ASPEN-POPULUS LINES IN RELATION TO STABLE OR UNSTABLE TRANSGENE EXPRESSION

M. Fladung, and S. Kumar

BFH, Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, Germany

mfladung@rrz.uni-hamburg.de  
<http://www.dainet.de/bfh/>

The stable expression of foreign genes in transgenic plants over their whole life span may be one of the difficult steps following transformation experiments. The prerequisites of stable expression of the foreign genes are: (i) their correct physical integration into the host genome, and (ii) their functionality. For long-lived tree species, however, a few informations are so far available on methylation or loss of transgenes due to gene silencing or somatic genome rearrangement events. Various independent transformed 35S-roIC transgenic aspen (*Populus tremula* L.) harbouring one to three copies of the transgene were investigated during their continuous growth in the greenhouse. Individuals in three of these transgenic lines frequently showed phenotypical back-reversions, while in the other lines high genetic stability of the gene was observed. Molecular analysis including PCR, Southern and northern experiments clearly showed that in the reverted tissue of the unstable lines either the transgene was present or had been lost. Analysis of the methylation patterns reveals a frequently increased methylation of some C-residues in two of the transgenic lines which may be responsible for the observed transgene inactivation. To increase the molecular understanding of the so-called 'position effect' T-DNA right and left border and flanking DNA regions of some transgenic aspen lines were sequenced. From various lines primer within the left and right flanking regions could be designed to recover plant DNA target sites from the untransformed plants. It was shown that various amounts of genomic DNA has been deleted following transgene integration.

**MONDAY, JULY 12, 1999**

**MOLECULAR ANALYSIS OF  
WOODY PLANT TRAITS**

University of Oxford Natural History Museum,  
Lecture Hall

## **SEMINAR 12**

### **GENE EXPRESSION DURING LOBLOLLY PINE EMBRYO DEVELOPMENT: TRANSCRIPT PROFILING AND GENE REGULATION STUDIES**

J. Cairney, N. Xu, J. MacKay, J. Pullman and X.Feng

Forest Biology Group, Institute of Paper Science and Technology, USA

john.cairney@ipst.edu  
<http://www.ipst.edu/>

The clonal propagation of high value forest trees from breeding or genetic engineering programs has the potential to help meet future industry needs by increasing forest yields and improving raw material uniformity. Somatic embryogenesis is a type of plant tissue culture which starts with a piece of donor plant and forms new genetically identical embryos, which develop in a sequence of changing tissue culture environments. Somatic embryogenesis holds great promise for commercial-scale clonal propagation however a major barrier to the commercialization of this technology is the quality of resulting embryos. Of major assistance in monitoring development, and improving somatic embryo quality, would be a series of markers for healthy development. Biochemical or genetic markers, however, have been difficult to obtain. We have used Differential Display to identify and isolate cDNA clones of genes that are expressed at different times during embryo development in embryo head and suspensor. Over 500 clones have been sequenced and gridded as DNA arrays. We are conducting 'transcript profiling' of these genes; hybridizing probes derived from RNA isolated from embryos at different stages of development. Our results establish a database for the quantity of mRNAs present for each of these differentially expressed genes over the course of embryo development. In addition to serving as markers, since many of these clones correspond to previously identified genes, these data give us insights into embryo biochemistry and physiology. The understanding gained from this work will shed new light on conifer embryogenesis and accelerate the improvement of somatic embryogenesis.

## SEMINAR 13

### ABA SIGNAL TRANSDUCTION DURING INDUCTION OF BUD DORMANCY IN POPLAR

A. Rohde, J. Van Doorselaere, M. Van Montagu, and W. Boerjan

Department of Plant Genetics, Flanders Interuniversity Institute for Biotechnology, University of Gent, Belgium

anroh@gengenp.rug.ac.be  
<http://www.plantgenetics.rug.ac.be>

The aim of our studies is to obtain new insight into the molecular processes underlying bud dormancy in trees. Previous physiological studies have suggested several parallels in the developmental programs of seed and bud dormancy. Asking whether both processes also share similar molecular mechanisms, we have studied the ABI (abscisic-acid insensitive) genes. In *Arabidopsis*, both ABI1 and ABI3 are required for the establishment of seed dormancy. Our previous work in *Arabidopsis* revealed that particularly ABI3 has additional functions during vegetative quiescence processes. We have isolated the homologous genes and cDNAs from *Populus trichocarpa*. By rtPCR, PtABI3 transcripts were detected exclusively in autumn buds, at about the time of vegetative growth arrest. At the same time, the expression of PtABI1 (another ABA signal transduction component) and PtVP14 (the gene encoding the key-enzyme of ABA biosynthesis) peaked. This result argues for a role of ABA and both PtABI3 and PtABI1 in the induction of growth arrest and bud set. Accordingly, *Populus tremula* x *P. alba* plants transformed with sense and antisense constructs of the PtABI3 gene exhibited an altered response to short-day-induced growth arrest. Besides this single gene approach, we compared the expression profiles in poplar buds throughout the induction of bud set (period from August to October) by cDNA-AFLP. Differentially expressed fragments were sequenced and their expression at bud set confirmed in independent experiments. Four fragments are being further characterized. This experiment strengthens the idea that an extensive transcript profiling could help to refine our knowledge on physiological processes and their interconnection during dormancy.

## SEMINAR 14

### **MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF A STRESS-INDUCED PINOSYLVIN-O-METHYLTRANSFERASE cDNA FROM SCOTS PINE (PINUS SYLVESTRIS L.)**

D. Ernst, H. Chiron, A. Drouet, W. Heller, and H. Sandermann

GSF-Institute of Biochemical Plant Pathology, Germany (DE, HC, WH, HS) and Laboratory of Plant Physiology, University of Orléans, France (HC, AD)

ernst@gsf.de  
<http://www.gsf.de>

Formation of pinosylvin and pinosylvin monomethylether, as well as the activities of stilbene synthase and S-adenosyl-L-methionine (SAM):pinosylvin O-methyltransferase (PMT), were strongly induced in needles of Scots pine seedlings upon ozone treatment, as well as in cell suspension cultures of Scots pine upon fungal infection. A SAM-dependent PMT was purified from elicitor-induced cell cultures and partially characterized with various stilbene substrates. Degenerated oligonucleotide primers based on a highly conserved consensus sequence of O-methyltransferases (OMT) and vector primers were used to isolate DNA fragments from an ozone-induced scots pine cDNA library by rapid amplification of cDNA ends (RACE). Additional PCR, using the start and stop regions of the RACE products resulted in a full-length PMT cDNA clone. The deduced protein sequence matched perfectly to a tryptic peptide sequence of the purified protein. It showed the typical highly conserved regions of OMTs. Amino acid sequence comparison with other OMTs showed average identities from 21% up to 58 %. The molecular mass of Scots pine PMT expressed in *E. COLI* (40 kDa) corresponded to that of purified PMT (40 kDa) pine cell cultures. The recombinant enzyme catalysed the methylation of not only pinosylvin, but also caffeic acid, caffeoylCoA and quercetin, with a lowest Km (14  $\mu$ M) towards pinosylvin. Kaempferol was not utilized. This indicates that PMT is a 3-OMT multifunctional enzyme. Southern blot analysis suggested the presence of a OMT gene family. Treatment of 7-year-old Scots pine trees markedly increase the PMT mRNA level, as shown by RT-PCR. PMT represents a novel SAM-dependent OMT for the methylation of stress-induced stilbenes in Scots pine needles.

## SEMINAR 15

### MOLECULAR APPROACHES TO PEST DEFENSE: CLONING AND CHARACTERIZATION OF HYBRID POPLAR AND TREMBLING ASPEN POLYPHENOL OXIDASE

C.P. Constabel, L. Yip, M. Haruta, and M. Christopher

Department of Biological Sciences, University of Alberta, Canada

[peter.constabel@ualberta.ca](mailto:peter.constabel@ualberta.ca)

<http://www.biology.ualberta.ca/constabel.hp/constabel.html>

In many plant species including trees, insect pest defenses are actively induced, that is, triggered by the herbivores. Such an induced defense response is characterized by rapid changes in gene expression which results in the increased synthesis and accumulation of defensive proteins and phytochemicals. In hybrid poplar (*Populus trichocarpa* x *P. deltoides*), as well as trembling aspen (*P. tremuloides*), leaf damage by forest tent caterpillar induces the accumulation of polyphenol oxidase. This enzyme oxidizes plant phenolics, which alkylate proteins and reduce the nutritive value of plant proteins for insect herbivores. PPO is an easily assayed marker for the induced defenses of poplar and aspen, and PPO induction was shown to be plant-wide in response to local leaf damage. PPO cDNAs were isolated from both hybrid poplar and aspen. Northern blot analysis shows that PPO induction occurs at the mRNA level. PPO is developmentally regulated in young leaves and other tissues, suggesting that it also acts as a constitutive defense. Experiments to overexpress PPO in transgenic trees are under way in order to demonstrate the role of PPO in defense. Other anti-nutritive proteins are also being characterized. A better understanding of induced defense mechanisms and identification of key genes will allow genetic engineering of insect resistance in *Populus*.

Supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.



## SEMINAR 16

### CHARACTERIZATION OF A METALLOTHIONEIN GENE FROM THE ACTINORHIZAL TREE CASUARINA GLAUCA

C. Franche, L. Laplaze, I. Bourrie, F. Guermache, F. Auguy, M. Rio, L. Constans, T. Frutz, E. Duhoux, and D. Bogusz

Physiologie Cellulaire et Moleculaire des Arbres, IRD-GeneTrop, France

franche@mpl.ird.fr

<http://www.mpl.ird.fr/biod.htm>

*Casuarina glauca* is an actinorhizal tree which forms a nitrogen-fixing symbiosis with an actinomycete, *Frankia*. In arid and semi arid regions with poor forest resources, *C. glauca* plays a major role in sustaining soil fertility and crop production, in controlling efficiently wind and soil erosion, and in providing fire wood. From a c-DNA library of *C. glauca* nodules, we isolated a sequence encoding a metallothionein (cgMT1). Metallothioneins (MTs) are a group of low-molecular weight cystein-rich proteins. In animals and fungi, MTs bind heavy metals such as zinc, cadmium and copper. MTs are believed to play a role in detoxification and metabolism of heavy metals through thiol binding. To better understand the role of cgMT1, a 1 kb fragment corresponding to the cgMT1 promoter was fused to the beta-glucuronidase reporter gene and the chimeric fusion was introduced into the actinorhizal tree *Allocasuarina verticillata* and in tobacco using *Agrobacterium tumefaciens*. In both plants, a strong reporter gene activity was seen in the roots and wound inducibility was observed in the aerial parts. In transgenic nodules of *A. verticillata*, GUS activity was restricted to nitrogen-fixing *FrankiaA*-infected cells, suggesting that the metallothionein might be involved in metal ions metabolism related to nitrogen-fixation. To further characterize cgMT1, the coding sequence was placed under the control of the 35S constitutive promoter and introduced into tobacco. Transgenic plants which were grown onto nutrient media containing different concentrations of heavy metals exhibited some tolerance towards cadmium and copper.

## SEMINAR 17

### AUXIN-INDUCED GENES FROM LOBLOLLY PINE AND THEIR ROLE IN DEVELOPMENT

B. Goldfarb, V.B. Busov and C. Lanz-Garcia

Department of Forestry, North Carolina State University, USA

goldfarb@cfr.cfr.ncsu.edu

Five auxin-induced genes (*LPEAs*), homologous to the *AUX/IAA* family in angiosperms, have been cloned from loblolly pine (*Pinus taeda* L.). Expression of these genes has been shown to be correlated with adventitious root formation, a developmental process that is stimulated by auxin, either from endogenous accumulation or exogenous application. Current studies show differences in expression magnitude and timing between juvenile cuttings that have the ability to form roots and mature cuttings that lack that ability. *LPEA* mRNA abundance is at a lower level and is more short-lived in mature cuttings as opposed to juvenile cuttings. Experiments to determine the regulation and function of the *LPEAs* are ongoing. A genomic clone for one of the *LPEAs* has been sequenced revealing the presence of several putative auxin-responsive cis elements in the promoter region. Different portions of the promoter have been fused to the GUS reporter gene and transformed into tobacco plants. Expression of the reporter gene is strongest in vascular tissue in leaves, stems and roots for all constructs. In roots, full-length promoter constructs cause expression in the vascular tissue of newly originated lateral roots, while partial promoter constructs cause loss of this expression pattern, but stimulate expression in root hairs, thus indicating a potential tissue-specific promoter element. Transformed tobacco plants, containing the full-length *LPEA* cDNAs fused with the CMV 35s promoter, have altered phenotypes. The most obvious phenotypes are reduced plant height, altered leaf shape and flower abnormalities. These effects appear to be gene-specific and may offer clues as to gene functions.

**TUESDAY, JULY 13, 1999**

**THE MOLECULAR BIOLOGY AND  
DIRECTED MODIFICATION OF WOOD**

University of Oxford Natural History Museum,  
Lecture Hall

## SEMINAR 18

### CELLULOSE – COVERING YOUR EMBARRASSING PARTS AND OTHER STORIES

D. Ellis

Forest Biotechnology Centre, BC Research, Canada

dellis@bcresearch.com

<http://www.bcr.bc.ca/forestry/default.htm>

Cellulose, one of the world's most abundant macromolecules making up a huge reservoir of carbon, also accounts for 90% of a plant cell's dry weight. And although its synthesis is an integral part of plant development and growth, little relative to other cell wall components, such as lignin, has been done with its genetic manipulation. Paradoxically, cellulose synthesis has been studied extensively in cellulose producing bacteria, most notably *Acetobacter xylinum*. The cell wall matrix of *A. xylinum* and plants is vastly different; however, the cellulose is composed of the same  $\beta$ -1,4-glucan chains. Therefore as a model, *A. xylinum* has proven invaluable in dissecting numerous biochemical and molecular processes involved in cellulose synthesis. In higher plants, for obvious reasons, cotton has been a focus for our understanding of factors regulating cellulose synthesis. In forestry, our reliance on cellulose is as fundamental as in cotton, however studies looking at modifying cell walls are very much in their infancy. In our lab, our interest in cellulose synthesis is to understand how carbon allocation and utilization within the plant can affect the synthesis of cellulose. These studies are aimed at determining the relationship between sink and source tissues, particularly as it relates to viewing the stem as a sink. These studies are focused on the relatively simple concept of increasing the level of cellulose precursors, however long-term goals include understanding and eventually manipulating the way cellulose is laid down in different cell wall layers, as well as altering linkages between cellulose microfibrils to increase growth and alter wood properties.

## SEMINAR 19

### A NOVEL XYLEM-SPECIFIC AND TENSION STRESS RESPONSIVE CELLULOSE SYNTHASE (PtCelA) GENE FROM QUAKING ASPEN

C.P. Joshi, L. Wu, V.L. Chiang

Plant Biotechnology Research Center, School of Forestry and Wood products, Michigan Technological University, USA

cpjoshi@mtu.edu

<http://forestry.mtu.edu/iwr/pbrc/personnel/cpjoshi/cpjoshi.html>

To understand the process of cellulose biosynthesis in trees, we cloned and characterized a full-length cellulose synthase cDNA (PtCelA) from quaking aspen. This cDNA is 3232 bp long and encodes a polypeptide of 978 amino acids with calculated molecular weight of 110 KDa and pI of 6.58. Significant amino acid sequence similarities exist between PtCelA and *Arabidopsis* RSW1 (71%) and cotton CelA genes (90%) that have been proposed to be authentic cellulose synthases. In situ localization of PtCelA transcripts in young aspen internodes demonstrated that the PtCelA gene expression is exclusively confined to the developing primary and secondary xylem cells undergoing secondary cell wall thickening. This cell-specific nature of PtCelA gene expression was also consistent with the xylem-specific activity of PtCelA gene promoter based on the heterologous PtCelA promoter-GUS fusion analysis. This is the first unequivocal demonstration of existence of a xylem-specific CelA in trees. PtCelA promoter also regulated phloem fiber-specific expression but only in response to tension stress. Such expression occurred rapidly within first four hours of stress. After 20 hrs of tension stress, both xylem and phloem exhibited GUS expression only in the upper half of the stem that was under tension stress but inhibited GUS expression in the lower half. With extended stress up to 40 hrs, GUS expression was restricted only to a small region on the upper side of the stem where maximum tension force was applied and GUS expression was turned off in all other tissues. These observations suggest that transcriptional regulation of CelA gene could be instrumental in the formation of tension wood cellulose in angiosperm trees.

## SEMINAR 20

### INCREASING POLYSACCHARIDE BIOSYNTHESIS VIA EXPRESSION OF CELLULOSE BINDING DOMAIN (CBD) IN TRANSGENIC PLANTS.

Z. Shani, E. Shpigel, M. Dekel, L. Roiz, and O. Shoseyov

CBD Technologies, Ltd., Israel (ZS, ES) and The Kennedy Leigh Center for Horticulture Research and The Otto Warburg Center for Agricultural Biotechnology, The Faculty of Agriculture, The Hebrew University of Jerusalem, Israel (MD, LR, OS)

[zshani@cbd-tech.com](mailto:zshani@cbd-tech.com)

Cellulose binding domains have been proved useful for the production and purification of many different CBD-fusion proteins. More recently it was shown that recombinant cellulose-binding domain (CBD) has modulated elongation of different plant cells *in vitro* (Shpigel et al., 1998, Plant Physiol. 117: 1185-1194). Using *Acetobacter xylinum* as a model system, CBD was found to increase the activity of the cellulose synthase enzyme in a dose-dependent manner, up to fivefold as compared with the control untreated cells. Expression of CBD gene under control of the different promoters led to significant increases in biomass production in selected clones as compared with the control. In addition, increased starch biosynthesis was observed in several instances. The mechanism of CBD in modulation of cellulose and starch biosynthesis will be discussed.

## SEMINAR 21

### REGULATION OF PLANT CELLULOSE BIOSYNTHESIS BY C-DI-GMP

R. Mayer, J. Kigel, and B. Rubin

Department of Agricultural Botany (RM, JK), and Department of Field Crops, Vegetables and Genetics (BR), Faculty of Agriculture, The Hebrew University of Jerusalem, Israel

mayer@agri.huji.ac.il

Research using the cellulose synthesizing bacterium *A. xylinum* has provided abundant and detailed information on its cellulose synthase. High rates of cellulose synthesis are dependent on the intracellular concentration of a novel and unique regulatory compound, c-di-GMP. The concentration of c-di-GMP - the net result of its rate of synthesis and rate of degradation - is controlled by the opposing action of two enzymes: Diguanylate cyclase (DGC), that catalyzes the synthesis of c-di-GMP from two molecules of GTP; and the c-di-GMP specific, phosphodiesterase A (PDE-A) that cleaves a single phosphodiester bond in the cyclic structure, yielding the inactive linear dimer pGpG. Thus far, there is no direct proof that c-di-GMP plays a role in plant cellulose biosynthesis. This possibility was examined by generating transgenic *Arabidopsis* plants that over-express the bacterial PDEA gene. The transgenic plant exhibited a subset of morphological phenotypes with respect to their response to the cellulose biosynthesis inhibitor, 2,6 dichlorobenzonitrile (DCB). To better understand the role played by DCB in cellulose biosynthesis, we tested whether it affects any of the enzymatic activities directly related to cellulose biosynthesis in bacteria. We found that DCB inhibited DGC activity. Taken together, these results suggest that PDE-A controls cellulose biosynthesis in plants and that the effect of PDE-A in *Arabidopsis* occurs when c-di-GMP concentration is limited by DCB.

## SEMINAR 22

### UNRAVELING LIGNIN BIOSYNTHESIS IN POPLAR

W. Boerjan, C. Chen, H. Meyermans, J. Christensen, K. Inoue, J.-C. Leplé, G. Pilate, C. Lapierre, B. Pollet, M. Petit-Conil and M. Van Montagu

VIB, University of Gent, Gent, Belgium (WB, CC, HM, JC, MVM), INRA-Orleans, France (J.-C.L, GP), INRA Grignon, France (CL, BP) CTP, Grenoble, France (MP-C), Samuel Roberts Noble Foundation, Ardmore, OK, USA (KI)

woboe@gengenp.rug.ac.be  
<http://www.vib.be>

Lignin is an abundant aromatic heteropolymer mainly present in cells that confer strength to the plant. Although the overall lignin biosynthesis pathway has been described for many years, it is still largely unclear what is the *in vivo* role of the different lignin biosynthesis enzymes in controlling the amount and composition of lignin in the cell wall, and which mechanisms determine lignin heterogeneity. These questions are relevant given the impact of lignin on paper making. To investigate the *in vivo* role of caffeoyl-CoA-O-methyltransferase (CCoAOMT) and cinnamoyl-CoA reductase (CCR), the expression of these genes was downregulated in transgenic poplar. Reduced expression of CCoAOMT resulted in a decreased lignin amount and into a reduced kappa number upon Kraft pulping. A reduced expression of CCR did not result in significant reductions in lignin content, although a reduced kappa number was observed upon simulated Kraft pulping. Peroxidases constitute a class of enzymes supposed to play a role in lignin polymerisation. We have purified from poplar xylem a number of anionic peroxidases putatively involved in lignin polymerisation, based on their ability to oxidise the monolignol analogue syringaldazine. Transgenic poplars have been generated with altered expression of one of these isoforms. Furthermore, to investigate the molecular basis of lignin heterogeneity, the cellular and subcellular expression of both caffeic acid-O-methyltransferase and CCoAOMT have been analysed by immunolocalisation experiments.

This work was carried out in the framework of the EU Research Programs AGRE-0021-C, FAIR-PL 95424, and AIR2-CT93-1661.



## SEMINAR 23

### ENABLING TECHNOLOGIES FOR THE COMPLEX MANIPULATION OF LIGNIN BIOSYNTHESIS

C. Halpin, A. Barakate, J. Abbott, B. Askari, A. O'Connell, W. Schuch and M.D. Ryan

Dept. Biological Sciences, University of Dundee, UK (CH, AB, JA, BA); Zeneca, UK (AO'C, WS) and University of St. Andrews, UK (MDR)

c.halpin@dundee.ac.uk

Just 5 years ago, we reported the first targeted genetic manipulation of a gene dedicated to monolignol synthesis. Enormous progress since then has meant that most of the genes on the pathway have now been manipulated in a variety of species, including trees, and field trials underpinning commercial exploitation are underway. Despite this, many basic aspects of the lignin pathway are still poorly understood. In addition, most work has concentrated on modulating the expression of single genes whereas full exploitation of the potential of genetic manipulation, both to investigate the pathway and to produce novel raw materials, depends on the ability to co-ordinately manipulate multiple genes. Our initial work was performed in tobacco, a herbaceous species that nonetheless becomes very woody at maturity. Tobacco is a particularly appropriate model and our results proved predictive of the effects of similar manipulations recently applied to poplar. Tobacco is also useful for developing generic technologies that might enable multiple gene manipulation in trees. We are currently using tobacco to develop strategies for the facile overexpression or downregulation of multiple lignin genes. For overexpression, proteins are encoded within a polyprotein that, on translation, self-dissociates into component polypeptides. For downregulation, partial sense sequences for up to three target genes have been fused and expressed from a single promoter. We have produced a library of tobacco mutants expressing altered levels of one, two or three lignin biosynthetic enzymes and are using them to further investigate the biochemistry and cell biology of lignification.

## SEMINAR 24

### NEW INSIGHTS ON LIGNIN ENGINEERING OF POPLARS: IMPACTS OF EXTREMELY LOW CAD AND OMT ACTIVITIES

L. Jouanin, T. Goujon, V. Ferret, G. Pilate, J.C. Leple, M. Noel, M. Petit-Conil, B. Pollet, I. Mila, C. Vallet, and C. Lapierre

Biologie Cellulaire, INRA Versailles, France (LJ, TG, VF), INRA CRO Orleans, France (GP, JCL, MN), CTP Grenoble, France (MP-C) Chimie Biologique INA-PG/INRA78850 Thiverval-Grignon, France (BP, IM, CV, CL)

jouanin@versailles.inra.fr

Lignin is a complex organic macromolecule which is responsible for cell wall properties important for tree development. However, it is an undesirable compound for the pulp and paper industry. Lignin structure affects the efficiency of industrial delignification processes. Therefore, in order to obtain trees with improved pulping performances, genetic engineering experiments have been targeted in poplar to genes coding for the enzymes caffeic acid O-methyltransferase (COMT) and cinnamyl alcohol dehydrogenase (CAD) involved in the biosynthesis of monolignols. We have recently obtained transgenic poplars (717 1B4, *Populus tremula* x *P. alba*) underexpressing COMT and CAD and characterized their specific lignin profiles and pulping behavior (1-3). A new 717 1B4 line under-expressing COMT was obtained. This line showed a substantial reduction in lignin content together with an increase in cellulose. In addition, a dramatic alteration of lignin structure was observed. A new construct containing the entire *Populus tricarpa* cad cDNA under the control of the CaMV 35S2 promoter was introduced in INRA 717 1B4 and in OGY (*Populus deltoides* x *P. nigra*) clones. This new construct induced the same structural changes in lignin as those previously reported (2,3) when introduced in the 717 1B4 background. In contrast, when introduced in the OGY background, the CAD down-regulation level and the alteration in lignin profile and reactivity were found outstandingly more pronounced. These results further demonstrate that a very important down-regulation of CAD and COMT is required to obtain an in-depth alteration of lignin content and structure.

1: Van Doorselaere et al, Plant J (1995) 8: 855-864.

2: Baucher et al (1996) Plant Physiol (1996) 112: 1479-1490.

3: Lapierre et al (1999) Plant Physiol 119: 153-163.

This work was carried out in the framework of the EU research program FAIR-TIMBER (CT95-0424).

## SEMINAR 25

### **CINNAMOYL CoA REDUCTASE, THE FIRST COMMITTED STEP OF THE LIGNIN BRANCH PATHWAY: EXPRESSION AND DOWN-REGULATION THROUGH ANTISENSE STRATEGY**

J. Grima-Pettenati, E. Lacombe, J Van Doorselaere, J. Piquemal , M. Chabannes, C. Lapierre, M. Petit Conil and AM Boudet

UMR n°5546 CNRS/ Université Paul Sabatier, France (JGP, EL, JP,MC,AMB), INAPG, France (CL), VIB, Belgium (JVD), CTP, France (MPC)

grima@cict.fr

Lignin constitutes a major component of the terrestrial biomass, accounting for up to 25-35% of the dry weight in woody species. This phenolic polymer plays a crucial role in plant development and defence responses. On the other hand, in the pulp and paper industry, the removal of lignin from cellulose is a costly and environmentally hazardous process. With the aim of improving the lignin « profiles » of commercially important forest species, we have employed antisense technology to down-regulate Cinnamoyl CoA Reductase (CCR) in a model plant, tobacco. The consequences of CCR down-regulation on lignin content and composition will be presented as well as the improvements of fibre characteristics for pulp making. In parallel, we examined the developmental regulation of the *Eucalyptus gunnii* CCR promoter by analysing the expression of CCR-GUS fusions in tobacco plants. CCR promoter expression is associated with lignified parts of the plant (stems and roots), consistent with the CCR mRNA level in these organs. Histochemical analysis showed expression in vascular tissues (cambium, young differentiating xylem, internal and external phloem and ray cells) of stems and roots. Promoter deletion analysis identified the sequences between position -121 and -79 as sufficient for expression in vascular tissues from stems and roots and root tips. This region specifically recognised by nuclear factors present in tobacco stems contains two DNA binding sites. One of them corresponds to the ACI-element found in other promoters of the phenylpropanoid pathway suggesting that MYB factors are involved in the transcriptional regulation of the CCR gene expression.

## SEMINAR 26

### PHENYLPROPANOID METABOLISM IN ARABIDOPSIS: BIOCHEMISTRY AND BIOTECHNOLOGY

C. Chapple , J.M. Humphreys, M.O. Ruegger, and M.R. Hemm

Department of Biochemistry, Purdue University, USA

chapple@biochem.purdue.edu

<http://www.biochem.purdue.edu/~chapple/>

We have identified nine loci in arabidopsis that affect the accumulation of phenylpropanoid secondary metabolites and lignin. The study of these mutants has provided new opportunities to isolate genes of the phenylpropanoid pathway, and use them for metabolic engineering in arabidopsis and other plants. For example, the *FAH1* locus of arabidopsis encodes the enzyme ferulate-5-hydroxylase (F5H) and mutations in this gene block the accumulation of syringyl lignin and sinapate esters. To characterize the enzyme in detail, we have expressed F5H in yeast. According to current models of the phenylpropanoid pathway, F5H catalyzes the hydroxylation of ferulate to 5-hydroxyferulate; however, our studies indicate that the enzyme more effectively uses coniferaldehyde and coniferyl alcohol as substrates. We have also demonstrated that all F5H reaction products are converted to their corresponding sinapyl derivatives by native or recombinant arabidopsis caffeic acid/5-hydroxyferulic acid O-methyltransferase. Taken together, these data suggest that the previously accepted pathway for lignin biosynthesis is likely to be incorrect. Finally, we have recently identified red fluorescent mutants representing five loci and several alleles of *brt1* (bright trichomes) in a saturation mutant screen for genes that decrease sinapoylmalate levels. Several of these genes are specific to sinapate ester synthesis, while others also alter lignin quality or quantity. Future work will be devoted to cloning the genes identified by these mutations to further our understanding of phenylpropanoid metabolism and its regulation.

## SEMINAR 27

### A CLARIFICATION OF SYRINGYL MONOLIGNOL BIOSYNTHESIS

L. Li, K. Osakabe, C.C. Tsao, J.L. Popko, T. Umezawa, D.T. Carraway, R.H. Smeltzer, C.P. Joshi, and V.L. Chiang

Plant Biotechnology Research Center, School of Forestry and Wood Product,  
Michigan Technological University, USA (LL, KO, CCT, JLP, TU, CPJ, VLC),  
International Paper Co., USA (DTC, RHS)

vchiang@mtu.edu

<http://forestry.mtu.edu/iwr/pbrc/>

<http://forestry.mtu.edu/iwr/forestbt.htm>

A paradigm which investigator in the field of lignin research have always adhered to is that the regulation of syringyl and guaiacyl lignin composition in angiosperms occurs at ferulate 5-hydroxylation step. However, this reaction, believed to be catalyzed by ferulate 5-hydroxylase (F5H), has never been unequivocally demonstrated. 5-Hydroxyferulate, the presumed F5H reaction product which has never been detected in lignifying xylem, has been considered as a substrate for caffeate O-methyltransferase (COMT) for the formation of sinapate to be further metabolized into syringyl monolignol sinapyl alcohol. As a norm, these enzyme activities, as well as those for other lignin pathway enzymes, were derived from in vitro enzymatic reactions using a single substrate, which does not take into consideration that multiple pathway intermediates must be present in the lignifying cell walls. Based on mass spectrometry-assisted product structural confirmation and enzyme kinetics, we now present conclusive evidence that ferulate 5-hydroxylation and 5-hydroxyferulate methylation do not occur in the presence of other pathway intermediates, and that there is no F5H per se. Coniferyl aldehyde completely inhibits ferulate 5-hydroxylation in a noncompetitive manner. In fact, coniferyl aldehyde is also the best guaiacyl intermediate for 5-hydroxylation to 5-hydroxyconiferyl aldehyde catalyzed by coniferyl aldehyde 5-hydroxylase (CAld5H) we now discover. While CAld5H reaction product 5-hydroxyconiferyl aldehyde is the best COMT substrate leading to the formation of sinapyl aldehyde, its presence inhibits competitively the methylation of caffeate and 5-hydroxyferulate, two traditionally accepted COMT substrates. Endogenous coniferyl, 5-hydroxyconiferyl and sinapyl aldehydes were also detected. Take together, these results are consistent with an in vivo operation of the CAld5H/COMT-mediated syringyl pathway from coniferyl to sinapyl aldehydes via 5-hydroxyconiferyl aldehyde. Thus, based on the previously identified substrate preference of 4CL, CCR, and CAD, these proteins together with CAld5H and COMT constitute a dynamic enzyme system that efficiently converts ferulate into coniferyl and sinapyl alcohols for the biosynthesis of guaiacyl-syringyl lignin in angiosperms.

## SEMINAR 28

### ENHANCED GROWTH AND CELLULOSE ACCUMULATION IN LIGNIN-REDUCED TRANSGENIC ASPEN WITH DOWN-REGULATED 4-COUMARATE:CoA LIGASE

C.-J. Tsai, S.A. Harding, W.-J. Hu, J. Lung, J.L. Popko, and V.L. Chiang

Plant Biotechnology Research Center, School of Forestry and Wood Products, Michigan Technological University, USA

chtsai@mtu.edu

<http://forestry.mtu.edu/iwr/pbrc/>

Lignin reduction in an economically important pulpwood species, quaking aspen (*Populus tremuloides* Michx.), has been successfully achieved by antisense down-regulation of a lignin pathway gene encoding 4-coumarate:CoA ligase (4CL). Severe (>90%) depletion of Pt4CL1 gene expression and enzyme activity resulted in up to a 45% reduction in lignin content and up to a 15% compensatory increase in cellulose content of the transgenic trees. Tree growth was substantially enhanced, featuring thicker stems, longer stem internodes, larger leaf size and faster root initiation. Increased leaf growth in transgenic trees is characterized by rapid and prolonged expansion of leaf epidermal cells. Phenolic profiles of the upper leaves and shoot apex are significantly altered in transgenic lines, reflecting possible secondary effects of Pt4CL1 down-regulation on non-lignin phenylpropanoid metabolism. Related to this is the observation of a pink coloration in the methanolic extracts of transgenic apices and leaves, perhaps indicating an increase in extractable flavonoids. This is consistent with our finding that phenylalanine ammonia-lyase (PAL) activity increased in leaves of transgenic plants. In addition, there is a stimulatory effect of the transgenic methanolic extracts on pea IAA-oxidase activity. Our data, therefore, indicate a regulatory role of 4CL in the coordination of growth, lignification, and phenolic metabolism. Our continuing investigation into the role of 4CL as it affects plant developmental processes pertaining to structural and defensive integrity, as well as cell division and growth will be discussed.

## SEMINAR 29

### GENETIC MODIFICATION OF THE LIGNIN BIOSYNTHETIC PATHWAY IN SPRUCE VIA DOWN-REGULATION OF THE CONIFERIN BETA-GLUCOSIDASE GENE

M. Gray-Mitsumune, M. Gilbert, L. A. Samuels, D. P. Dharmawardhana, C. J. Douglas, J. E. Carlson, D. D. Ellis and B. E. Ellis

Department of Plant Science, University of British Columbia, Canada (MG-M, BEE), Forest Biotechnology Centre, BC Research Inc. (MG, DPD, DDE), Biotechnology Laboratory, University of British Columbia, Canada (MG-M, CJD, JEC, BEE), Department of Botany, University of British Columbia, Canada (LAS, CJD), and School of Forest Resources, Pennsylvania State University, USA (JEC)

madokag@interchange.ubc.ca  
<http://www.agsci.ubc.ca/plantsci/index.html>

Coniferin beta-glucosidase (CG) is involved in the last steps of the lignin biosynthetic pathway by releasing the lignin precursor, coniferyl alcohol, from its glucoside form, coniferin, prior to polymerization in the cell wall. We have previously cloned a cDNA sequence of CG from lodgepole pine. CG was predicted, from its deduced amino acid sequence, to be extracellularly localized. This was supported by our preliminary result with immuno-gold staining of developing xylem using polyclonal antibody against CG. Our hypothesis is that coniferin serves as an intracellular transport form of coniferyl alcohol from the cytosol to the cell wall. Thus, release of its aglycone in the cell wall is likely be an essential step in lignin biosynthesis *in vivo*. We have taken a transgenic approach to gain further insight into the role of CG in lignin biosynthesis. An antisense construct against the CG gene under control of the cauliflower mosaic virus 35S promoter was prepared using the lodgepole pine CG cDNA sequence. Interior spruce was chosen as target species because of its well-established regeneration system. Somatic embryos of interior spruce were transformed with the antisense CG gene using a particle gun and selected on kanamycin-containing medium. Fifty-seven lines have been confirmed to be transformants, as detected by PCR analysis. Among them, 46 lines carried the antisense CG gene and 11 lines carried only marker genes. These cell lines have been regenerated into plants via somatic embryogenesis. These are now growing in a greenhouse in preparation for phenotypic analyses.

## SEMINAR 30

### CHARACTERIZATION AND EXPRESSION OF LACCASE GENES FROM YELLOW-POPLAR XYLEM

J.F.D. Dean, P.R. LaFayette, J.T. Hoopes, and B.C. McCaig

School of Forest Resources, University of Georgia, USA

jeffdean@uga.edu

<http://www.arches.uga.edu/~jeffdean/JDeanUGA.html>

Four cDNA clones encoding laccase isoenzymes expressed in lignifying xylem of yellow-poplar (Ltlacc2.1-4) were identified and sequenced. The inferred yellow-poplar laccase gene products were highly related to one another (79-91% at the amino acid level) and showed significant similarity to other blue copper oxidases, especially with respect to the copper-binding domains. The encoded proteins had N-terminal signal sequences and 17-19 potential N-linked glycosylation sites. The mature proteins were predicted to have molecular masses of ca. 61 kDa (nonglycosylated), high isoelectric points (pI 9.3 - 9.5). The canonical copper ligands were completely conserved, with the exception of a Leu residue associated with the axial position of the Type-1 cupric ion. The residue at this position has been proposed to influence the redox potential of Type-1 cupric ions. Northern blot analysis revealed that the yellow-poplar laccase genes are differentially expressed in lignifying xylem. The genes were verified as encoding active laccases by heterologous expression in tobacco cells and demonstration of laccase activity in extracts from transformed tobacco cell lines. The four yellow-poplar laccase genes form a single monophyletic unit when compared to all other laccases whose sequences are available in the public databases. Various portions of this work were supported by grants from the U.S. Department of Energy (DE-FG05-95ER20182), the Georgia Traditional Industries Program - Pulp and Paper (PP96-FS3), and an NSF/Alfred P. Sloan Foundation Postdoctoral Research Fellowship in Molecular Evolution (DBI-9803949) awarded to BCM.



## SEMINAR 31

### ARABIDOPSIS THALIANA: A MODEL PLANT FOR LIGNIFICATION STUDIES

L. Jouanin, T. Goujon, V. Ferret, B. Pollet, C. Lapierre

Biologie cellulaire, INRA Versailles, France (LJ, TG, VF) and Chimie biologique INA-PG/INRA, France (BP, CL)

jouanin@versailles.inra.fr

Lignins are complex polymers that are deposited in cell walls of tissues involved in mechanical support or water conduction. The lignin biosynthesis pathway has not been totally understood so far. Our aim is to use *Arabidopsis thaliana* as a model plant to study lignification as an alternative to woody species. Genes of the biosynthesis pathway of monolignols (monomeric units) were identified in EST databases and the tissue-specificity of their expression was determined in *A. thaliana*. *A. thaliana* plants carrying antisense constructions for cinnamyl alcohol dehydrogenase and cinnamoyl-CoA reductase were obtained and characterized. CCR antisense plants possess characteristics similar to CCR antisense tobacco plants (Piquemal et al, 1998. Plant J 13: 71-83) The screening of the Versailles T-DNA insertion mutant *A. thaliana* library has been initiated. The inserted T-DNA contains, near the right border, a beta-glucuronidase (GUS) gene without promoter sequences. 5 lines (among 4000 screened lines) expressing GUS activity in the xylem have been identified. The characterization of some of these mutants will be reported.

## SEMINAR 32

### TOBACCO TRANSCRIPTION FACTOR NTLIM1 REGULATES LIGNIN BIOSYNTHESIS

A. Kawaoka, and H. Ebinuma

Central Reserach Laboratory, Nippon Paper Industries, Japan (AK,HE)

akiyoshi.kawaka@nifty.ne.jp  
[http://www.npaper.co.jp/e\\_html/index.html](http://www.npaper.co.jp/e_html/index.html)

Lignin is a major component of wood and must be degraded to extract cellulose fibers for paper making. Genetic engineering of lignin biosynthesis in pulpwood has potential to produce new woody plants with altered lignin content and composition. To know the regulation of lignin biosynthesis, we focused on a transcription factor that controls gene expression in this pathway. The AC-rich motif, Pal-box is thought to be an important cis-acting element for gene expression involved in phenylalanine biosynthesis from functional analysis of promoter regions. We prepared the cDNA expression library from tobacco stem. A cDNA clone (Ntlm1) that encodes the Pal-box binding protein, was isolated by Southwestern screening method. The deduced amino acid sequence has similarity to a LIM protein family. The LIM motif defines one class of zinc-binding domain and was originally recognized in the protein products of the lin-11, isl-1 and mec-3 genes. The gene products of lin-11 and mec-3 transcriptionally regulate genes involved in cell fate determination and differentiation in *Caenorhabditis elegans*, and the isl-1 gene encodes a rat insulin I gene enhancer-binding protein. The Ntlm1 activated the transcription of beta-glucuronidase reporter gene driven by PAL-box sequence in tobacco protoplasts. Transgenic tobacco with antisense Ntlm1 showed the low expression levels of phenylalanine ammonia-lyase, 4-coumaroyl-CoA ligase and cinnamyl alcohol dehydrogenase. Furthermore, the lignin content in the transgenic tobacco with antisense Ntlm1 decreased to 70% as compared with that in control plants.

## SEMINAR 33

### WOOD STRUCTURE AND CELL WALL ARCHITECTURE IN RoIC TRANSGENIC ASPEN TREES

C. Grünwald, U. Schmitt, F. Deutsch, M. Fladung, and D. Eckstein

Institute for Wood Biology, University of Hamburg, Germany (CG, US, FD, DE) and Institute for Forest Genetics, Federal Research Centre for Forestry and Forest Products, Germany (MF)

gruenwald@aixh0401.holz.uni-hamburg.de  
<http://www.dainet.de/bfh/indexe.htm>

The control mechanisms in xylogenesis, although widely discussed in literature, are not yet fully understood. Therefore the mostly accepted auxin-hypothesis was tested using 35S-ROLC-transgenic aspen trees, which are characterised by dwarfed phenotype and a precocious bud as well as leaf development as compared to untransformed controls. It could be shown that the early bud break does not lead to an earlier activation of the cambium in the transgenics. In the controls, however, there was a close connexion between bud break and cambium activation. In order to investigate the impacts of the ROLC gene on wood-biological traits, wood structure of 35S-ROLC-transgenic aspen trees was analysed. Histometric measurements did not show significant differences, but some qualitative features were distinctive in the transgenics, above all an atypical latewood formation, characterised by thin-walled, less lignified and even disarranged fibres. Ultrastructural analyses revealed unusual cell wall layering - in some cases wall layers were missing, in other cases adjacent wall layers were not completely adjoined. These structural defects as well as chemical alterations during cell wall development probably are responsible for disarrangements of cells in the tissue as well as abnormal shapes and sizes of individual cells. These described differences in cell wall architecture make 35S-ROLC-transgenic aspen trees a useful system to study regulation mechanisms of wood formation. The aim of future investigations is to compare the process of wood formation between 35S-ROLC-transgenic and control trees in order to find genes involved in xylogenesis and to characterise them by physical, chemical and biological analyses.

**WEDNESDAY, JULY 14, 1999**

**FROM LAB TO FIELD:  
THE APPLICATION OF FOREST TREE  
MOLECULAR BIOLOGY AND WOOD  
BIOTECHNOLOGY IN FORESTRY**

University of Oxford Natural History Museum,  
Lecture Hall

## **SEMINAR 34**

### **THE CASE AGAINST GENETIC ENGINEERING IN FARMING AND FORESTRY**

P. Holden and M. Wenban-Smith

The Soil Association, UK

mwenbansmith@SoilAssociation.Org  
<http://www.SoilAssociation.Org>

The Soil Association promotes the adoption of responsible and sustainable forest practices. To accomplish this, the Soil Association has developed a fully integrated forestry programme. Through the Responsible Forestry Programme, it plays an active part in developing systems of sustainable and responsible forest management and helps to establish them. It assists countries and forest enterprises to develop their own guidelines and standards of good forest practice. Through the Woodmark certification scheme, the Soil Association certifies and labels timber and wood products from well managed forests world-wide. Woodmark is fully accredited by the Forest Stewardship Council.

This talk will be divided into two parts. Patrick Holden, Director of the Soil Association will present the Soil Association's general position against genetic engineering. Mathew Wenban-Smith will then develop that argument with specific reference to forestry.

## SEMINAR 35

### OUTCROSSING RISKS FOR TRANSGENIC HYBRID POPLARS

S. DiFazio, S. Leonardi, S. Cheng, S. Garman, W.T. Adams, and S.H. Strauss

Oregon State University, USA

difazios@fsl.orst.edu

<http://www.fsl.orst.edu/tgerc/index.htm>

Risk assessment for transgenic trees presents special challenges because of the space and time scales involved, but also because of the complex interplay of ecological, genetic, agronomic, and social factors. We are assessing potential risks of largescale cultivation of fertile transgenic hybrid poplars (*Populus spp.*) in the Northwestern United States. To predict gene flow from transgenic plantations, it is necessary to understand both the dynamics of gene movement within and between populations, and the ability of hybrid trees to produce fit progeny. We have directly measured gene movement from non-transgenic hybrid plantations and in wild populations using molecular markers and paternity analysis methods. We have also assessed establishment and competitiveness of hybrid seedlings in the wild. Using data from these gene flow studies, field trials, remote sensing databases, and the literature, we are developing a spatially explicit computer model to simulate transgene spread from plantations in the Pacific Northwest of the United States. The model will be used to identify the genetic and ecological parameters most critical to predicting the spread of herbicide and insect resistance transgenes over space and time. We will assess outcomes of the model and field studies in the context of the agronomic and ecological settings where the trees will be grown, based in part on a formal survey of land management professionals.

## SEMINAR 36

### **TRANSGENIC TOBACCOS WITH DEPRESSED OMTs HAVE REDUCED LIGNIN CONTENT, MODIFIED VASCULAR ORGANISATION AND WEAKENED ANTI-VIRAL RESISTANCE**

S. Maury, L. Hoffmann, G. Pinçon, P. Geoffroy, R. Bronner, C. Lapierre, B. Fritig and M. Legrand

IBMP du CNRS, France

stephane.maury@ibmp-ulp.u-strasbg.fr

Tobacco is an useful model plant to study the regulation of phenylpropanoid metabolism and its role in resistance towards pathogen. Phenylpropanoid pathway provides the building units of lignin and many other phenolics that are incorporated into the plant cell wall. Two methylation steps are involved in the biosynthesis of monolignols, yielding guaiacyl and syringyl units of lignin. A first methyl group is introduced at the level of caffeoyl-CoA by caffeoyl-CoA 3-O-methyltransferases (CCoAOMTs), leading to feruloyl-CoA. A second methylation gives rise to sinapic acid from 5-hydroxyferulic acid and is catalysed by caffeic acid 3-O-methyltransferases (COMTs). In tobacco, we have characterised three classes of CCoAOMT genes and two classes of COMT genes, based on differences in sequences, expression profiles during stem development and virus infection and substrate specificities of encoded proteins. By antisense RNA expression, we have produced transgenic tobacco plants repressed in CCoAOMTs or COMTs or both. Transformant analysis revealed important reduction in total lignin amount and a marked decrease of growth rates. Histological studies showed a disorganisation of vascular tissues of stems, leaves and petioles. Transgenic plants also displayed a weakening of the level of virus resistance measured by necrotic lesion size, accompanied by profound changes in soluble and cell wall bound phenolics.

## SEMINAR 37

### RELEASING WILD CHERRY CLONES TO THE UK FORESTRY INDUSTRY- THE CHALLENGE

N. Hammatt, K. Russell and KR Tobutt

Horticulture Research International

Neil.Hammatt@hri.ac.uk

<http://www.hri.ac.uk/site2/woodland.htm>

Since 1988, we have been identifying superior, native wild cherry (*Prunus avium* L.), and breeding for fast growth, good form, and resistance to cherry blackfly and bacterial canker. In 1998, we selected the first ten clones, with good vigour and form and with varying levels of resistance to canker, for release to the UK industry. With funding from the Ministry of Agriculture, Fisheries and Food for technology transfer, HRI has used a variety of methods (articles in trade and general press, attendance at trade shows, an internet web site and technical advice leaflets) to inform the many different sectors of the industry. Though time consuming and at times expensive, feedback from the industry has been positive and valuable. Formation of the British Hardwoods Improvement Programme consortium in 1997 has aided this process by encouraging better co-ordination of research and industry. Factors affecting commercialisation include guidelines on the number of clones and their maximum 'shelf-lives', and the proportion of this species acceptable (currently 15%) in woodland plantings. To ensure planting material of uniform high quality, specifications including micropropagation and subsequent handling have been supplied to the propagators. A price premium over currently available unimproved seedlings may be resisted by some potential customers; thus, good marketing and economic justifications will be essential. Demonstration plots have been established with forestry organisations and landowners to show the superiority of new clones. A clonal seed orchard has been planted to supply cheaper seedlings of known UK origin; these will not be available for several years.



## **SEMINAR 38**

### **FORESTRY BIOTECHNOLOGY JOINT VENTURE**

David Duncan

Monsanto, USA

Fletcher Challenge Forests, International Paper, Monsanto Company and Westvaco Corporation announced their intent to form a forestry biotechnology joint venture to produce and market tree seedlings that will improve forest health and productivity for the forestry market worldwide. The four companies will contribute \$60 million (US) in total over five years to the joint venture and provide the business with substantial biotechnology resources to begin operation. The companies also announced their intent to contract with Genesis Research and Development Corporation Limited, an Auckland, New Zealand, biotechnology research company, to provide genomics research. The joint venture also will acquire forestry intellectual property from Genesis. The participating companies envision the joint venture as a worldwide magnet for future developments in forestry biotechnology. Plans call for the joint venture to actively seek technological advances from independent laboratories, universities and other companies. The four companies believe that as international demand for wood fiber increases, significant business opportunities will result from additional breakthroughs in forestry science. Each company possesses significant biotechnology capabilities and will share its individual strengths as an equal partner in the joint venture. As a result, the joint venture will be positioned to market new advances in forestry biotechnology to the world's tree growers in the shortest time. The joint venture will focus its efforts on tree species that represent a majority of the seedlings now planted by the forest industry around the world. The joint venture's initial efforts will involve various eucalyptus and poplar species, Radiata pine, loblolly pine and sweetgum. Targeted genetic improvements include:

- herbicide tolerant planting stock to enable more cost effective, as-needed control of competing vegetation;
- higher growth rates to allow more wood to be grown on less land at lower cost;
- improved fiber quality and uniformity to increase efficiency in paper and wood products manufacturing processes.

These improvements are expected to enable forest landowners to meet the growing demand for paper and wood products while strengthening their ability to manage forestlands in a sustainable and eco-efficient manner for the benefit of future generations. Increasing the productivity of tree plantations safely and sustainably will help meet the world's wood and fiber needs without increasing pressure on native forests.

## SEMINAR 39

### GENETICALLY ENGINEERED TREES FOR COMMERCIAL FORESTRY

J.A Charity, S. Bishop-Hurley, L. Donaldson, L.J. Grace, R. Moyle, A. Wagner, C. Walter

Genetic Engineering for Future Forests, Forest Research, New Zealand

[julia.charity@forestresearch.co.nz](mailto:julia.charity@forestresearch.co.nz)

<http://www.forestresearch.cri.nz>

In the near future, plantation forestry in New Zealand is well poised to gain considerable benefits from genetic engineering of forest trees. *Forest Research* has developed an efficient transformation protocol, using biolistic transformation of embryogenic tissue, to transfer a range of desirable traits into conifers. Transgenic *Pinus*, *Picea* and *Abies* species have been regenerated and trees are growing in either field trials or containment glasshouses. For example, transgenic *Pinus radiata* and *Picea abies* plants have been produced that are resistant to operational doses of herbicide phosphinothricin. Research is underway to provide a stable platform of robust transformation technologies for forest trees including both *Agrobacterium*-mediated techniques as well as biolistics. Early screening protocols such as the genetic transformation of cell cultures producing tracheary elements (currently in development) will allow us to test the function of novel genes in a rapid and cost-effective manner. In the long-term interest of the forestry sector, *Forest Research* aims to better understand fundamental developmental pathways such as those involved wood formation and reproductive development. To this end, genes and promoters involved in lignin biosynthesis, cellulose deposition, flowering and other developmental processes have been identified and isolated. *Forest Research* has forged links with the forestry sector including industry and research partners to provide a range of services encompassing tissue culture techniques, transformation technologies, gene and promoter discovery and propagation and deployment. The commercialisation of genetically engineered forest trees with traits such as herbicide, insect or pest resistance, altered wood qualities or modified reproductive characteristics, will rapidly become a reality in New Zealand.

## SEMINAR 40

### GENE FLOW FROM TRANSGENIC PLANTATIONS: PREVENTION AND ANALYSIS

S. Strauss, A. Brunner, J. Skinner, R. Meilan, S. DiFazio, and S. Leonardi.

Department of Forest Science, Oregon State University, USA

strauss@fsl.orst.edu

<http://www.fsl.orst.edu/tgerc/index.htm>

Major foci of work in our laboratory during the last decade has been on the prevention of release of transgenes into the environment from genetically engineered trees, and assessment of the likely impacts of release were fertile transgenic clones deployed. We focus on hybrid poplars grown under intensive culture in the Pacific Northwest of the United States, for which we have produced transgenic versions of elite clones that are nearly ready for commercial deployment. The need for solutions to the problem of transgene release are therefore immediate. In addition, recent controversy over transgenic crops in Europe and other countries, and the increase of third-party green certification systems for forest products, suggest that a system for strict containment of transgenes will be necessary for many genes and in many parts of the world. We have taken several approaches to genetic engineering of sterility. Some key findings are: (1) several heterologous sterility inducing transgenes, all of which contain floral promoter::cell toxin fusions, cause substantial reductions in tree growth rate despite having no apparent effects on morphology. (2) A number of genes reported to induce precocious flowering in herbaceous species gave no effect, or an inconsistent one, in transgenic poplars. (3) The structure and expression of tree homologs often create complications for engineering sterility via either promoter::cytotoxin or gene suppression methods. Our analyses of gene escape include studies of gene flow from hybrid plantations and wild stands, and prediction of transgene impacts and extent via spatial simulation modelling.

**WEDNESDAY, JULY 14, 1999**

**THE MOLECULAR BIOLOGY OF  
FLOWERING IN TREES**

University of Oxford Natural History Museum,  
Lecture Hall

## SEMINAR 41

### REGULATION OF FLOWERING TIME IN TREES

O. Nilsson

Dept. of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Sweden

Ove.Nilsson@genfys.slu.se  
<http://www.genfys.slu.se/>

The flowering of *Arabidopsis thaliana* is regulated by three classes of genes acting in consecutive order: the flowering time genes, the meristem identity genes and the flower organ identity genes. The function of the meristem identity gene *LEAFY* is conserved between species as diverse as *Arabidopsis*, tobacco and aspen. Furthermore, functional conservation has been shown for the organ identity gene *AGAMOUS* between spruce and *Arabidopsis*. However, it remains to be demonstrated that the flowering time genes have a function outside the Brassicaceae. We will discuss advantages and disadvantages of using ectopic *LEAFY* expression as a tool to control the flowering time of trees, and present experiments aimed at understanding the role of flowering time genes in the flowering of hybrid aspen. Finally, we will discuss how data from the *Populus* EST project can be used to identify genes important in the regulation of *Populus* flowering.

## SEMINAR 42

### **GENES CONTROLLING FLOWERING IN POPULUS: HOMOLOGS OF THE ARABIDOPSIS GENES APETALA1 (AP1) AND AGAMOUS (AG)**

A. Brunner, K. Krutoviskii, S. DiFazio, S. Leonardi, and S. Strauss

Department of Forest Science, Oregon State University, USA

brunnera@fsl.orst.edu

<http://www.fsl.orst.edu/tgerc/index.htm>

An ultimate goal of our research is to enable the manipulation of flowering (promotion, inhibition, and gender determination) in order to accelerate the rate of genetic improvement and to mitigate unintended ecological effects that could result from cultivation of transgenic trees. Towards that end, we have isolated *Populus trichopulus* homologs of floral homeotic genes and flowering time genes. These include two AP1 homologs (PTAP1-1, PTAP1-2) and two AG homologs (PTAG1, PTAG2). Phylogenetic and expression studies suggest that the poplar gene pairs were the result of relatively recent duplications of an AP1 and AG ortholog. Using SSR markers, PTAG1 and PTAG2 were mapped to separate linkage groups; mapping of PTAP1 genes is underway. Poplar flowers are atypical, consisting of an outer whorl organ, the perianth cup, and an inner whorl of either stamens or carpels. PTAG1 and PTAG2 exhibit an AG-like floral expression pattern consistent with a function in specifying reproductive organ identity. Unlike AG, however, vegetative expression is readily detectable for both genes. Initially, PTAP1-1 and PTAP1-2 are expressed throughout the emerging floral meristems, suggesting that they share functional orthology with AP1 in specifying floral meristem identity. Surprisingly, however, their expression is not maintained in perianth cup primordia, indicating that they do not specify its identity as would be expected from their hypothesized homology to sepals and petals. The unusual expression of PTAP1 genes in poplar may be a cause of its distinctive floral morphology.

## SEMINAR 43

### ENGINEERING OF CYTOTOXIN-BASED GENETIC STERILITY IN POPLAR USING THE PROMOTER OF A DEFICIENS-LIKE GENE FROM POPULUS TRICHOCARPA

J.S. Skinner, C. Ma, S. Cheng, R. Meilan, and S.H. Strauss

Tree Genetic Engineering Research Cooperative, Department of Forest Science, Oregon State University, USA

skinnerj@fsl.orst.edu

<http://www.fsl.orst.edu/tgerc/index.htm>

The PTD gene, a member of the DEF/TM6 class of MADS box floral homeotic genes, was previously isolated in our laboratory (1) from *Populus trichocarpa* (black cottonwood). PTD gene expression was observed in early and late male and female inflorescences by gel-blot and in situ expression analysis. Expression was not detected in roots, vegetative buds, or seedlings. These results suggested that the PTD promoter might be useful for engineering of male and female sterility by cytotoxic ablation of floral structures. To test the suitability of the PTD promoter for engineering sterility, a 1.9 kb genomic fragment containing the upstream regulatory region was isolated and used to direct the expression of the GUS reporter gene in transgenic *Arabidopsis*, tobacco, and early-flowering poplar (poplar co-expressing 35S::LFY). In *Arabidopsis* and tobacco, histochemical GUS expression was observed in petals, stamens and at the base of flowers; vegetative expression was not observed. In early-flowering poplar, reporter gene expression was observed in induced floral-like structures, including the floral structure base. In vegetative tissues, expression was only observed in leaves directly subtending induced floral structures. Based on these results, the PTD promoter fragment was used to direct expression of the cytotoxin DTA in *Arabidopsis*, tobacco, and early-flowering poplar. In *Arabidopsis*, preliminary results have demonstrated petal ablation and stamen perturbation. In early-flowering poplar, induced floral structures fail to develop in >90% of poplar lines containing both PTD::DTA and 35S::LFY transgenes.

(1) Sheppard, L. 1997. PTD, a floral homeotic gene from *Populus trichocarpa* with homology to transcription factors. PhD thesis, Oregon State University, Corvallis, USA.

## SEMINAR 44

### TOWARDS NON-FLOWERING BIRCHES

J. Lemmetyinen, M. Lännenpää, A. Elo, I. Porali, P. Hyttinen, K. Keinonen-Mettälä, and T. Sopenen

Department of Biology, University of Joensuu, Finland

tuomas.sopenen@joensuu.fi

<http://www.joensuu.fi/matemluonto/biologia/sopenen/sopenen.html>

Silver birch (*Betula pendula* Roth) is one of the economically very important trees in Finland and elsewhere in the temperate region. Birch can be micropropagated and transformed, and therefore attempts to use genetic manipulation in birch breeding have been started e.g. in Finland. In order to prevent the spreading of the transgenes during testing and cultivation, we have aim at inducing sterility in birch. Sterility might also increase the growth of the trees. Our approach is to use an inflorescence-specific promoter to induce expression of a cytotoxin gene in the developing inflorescence. This should lead to death of the developing inflorescence and thus to sterility. For this purpose we are studying genes regulating flower development in birch. So far we have isolated 9 cDNAs from birch, which on the basis of homology, regulate flower development. These include homologues of APETALA1, AGAMOUS, PISTILLATA, LEAFY, AGL8, AGL9, AGL11, and SBP1. Some of these genes are expressed only in the inflorescences, some are expressed also in some vegetative tissues. The promoters of four of these genes have been isolated and the expression of two promoter:: GUS constructs have been studied in transgenic tobacco and are being studied in early-flowering birch lines. The results of these studies will be reported.



## SEMINAR 45

### GENETIC REGULATION OF FLOWERING: EXPLORING POTENTIAL APPLICATIONS TO MANIPULATING CONE FORMATION IN CONIFERS

R.G. Rutledge

Canadian Forest Service, Natural Resources Canada, Canada

brutledge@cfl.forestry.ca  
<http://www.cfl.forestry.ca>

Mutant analysis combined with transgenic manipulation has provided an unprecedented understanding of the genetic pathways controlling phase change, flower initiation and flower development. Recent characterisation of homologues of *LEAFY* and *AGAMOUS* from pine and spruce respectively, suggests that at least some of the major regulatory mechanisms controlling sexual reproduction in conifers are similar to that of angiosperms. Demonstration that these conifer genes are functional within transgenic arabidopsis provides direct supporting evidence for this, and suggests that flower regulatory genes from angiosperms may be functional within transgenic conifers. The potential to modify cone initiation and development in transgenic conifers using a reverse genetics approach could help to circumvent some of the experimental limitations inherent to conifers, and allow exploration into the genetic pathways regulating conifer reproduction. As a first step towards this goal we have generated large numbers of transgenic lines of black spruce constitutively expressing Arabidopsis *LEAFY* or the black spruce *AGAMOUS* homologue, *SAG1*. Embryonal tissue and somatic embryos show a normal phenotype even in lines expressing high levels of either of these transgenes. Transgenic seedlings, some of which have been maintained under accelerated growth conditions for over two years, have to date not produced any phenotypes that could be directly correlated with transgene activity, other than a propensity to set bud. Although these trees have yet to reach sexual maturation, these data suggest that the dramatic effects of *AGAMOUS* and *LEAFY* ectopic expression on juvenile growth in transgenic Arabidopsis are absent or much reduced in young transgenic spruce trees.

## SEMINAR 46

### MOLECULAR DISSECTION OF PHASE CHANGE IN EUCALYPTUS

A.J.Collins, K.Meyer and M.M.Campbell

Department of Plant Sciences, University of Oxford, UK (AJC, MMC) and Shell Forestry Ltd, UK (KM)

angela.collins@plants.ox.ac.uk  
<http://users.ox.ac.uk/~dops0022>

The *TERMINAL FLOWER1* (*TFL1*) gene from *Arabidopsis thaliana* has been shown to affect the rate of maturation (Ratcliffe *et al.*, 1998). The *TFL1* mutant flowers early and produces terminal flowers in place of indeterminate inflorescences. The presumed orthologue from *Antirrhinum majus*, *CENTRORADIALIS* (*CEN*) has no effect on timing of flowering, but, *cen* mutants do produce terminal flowers (Ratcliffe *et al.*, 1998). We are investigating molecular determinants of phase change in *Eucalyptus* sp. Towards this end, we have focused our attention on isolating the eucalyptus orthologues of *TFL1/CEN*. Using degenerate PCR, eucalyptus *TFL1/CEN* orthologues have been cloned. The eucalypt orthologues have a very high identity with the arabidopsis/ *Antirrhinum* genes. The PCR-generated eucalyptus *TFL1/CEN* orthologues have been used to isolate genomic clones of the corresponding eucalyptus *TFL1/CEN* genes. These genes are being used in complementation tests of the *tfl1-14* mutant.

Ratcliffe *et al.*, (1998) *Development* 125: 1609-1615.

## **SEMINAR 47**

### **OVULAR SECRETIONS IN SEED PLANTS: EVOLUTION AND BIOCHEMICAL COMPLEXITY**

Patrick von Aderkas

University of Victoria, Canada

[pvonader@uvvm.uvic.ca](mailto:pvonader@uvvm.uvic.ca)

<http://darwin.ceh.uvic.ca/people/vonaderk.html>

Ovule secretions from lagenostomalean seed plants to angiosperms can be categorized according to the timing of their appearance. The origin of the ovular secretions may be from either the nucellus, the integuments, or from the megagametophyte. The evidence for a nucellar origin will be considered in terms of its structural evolution. Recent work on ovular secretions in angiosperms and gymnosperms has shown that these solutions are more complex than previously thought. Advances in microanalytical techniques and molecular biology are providing opportunities to better characterize the structure and role of the diverse compounds found in these drops. The occurrence of proteins and carbohydrates, as well as a other classes of compounds in postpollination/prefertilization drops of larch and Douglas fir will be discussed.

## SEMINAR 48

### FLOWERING AND SPIKELET PROLIFERATION OF *BAMBUSA EDULIS* IN VITRO

W.C.Chang, and C.S.Lin

Institute of Botany, Academia Sinica, Taiwan

wcchang@wcc.sinica.edu.tw

A protocol for somatic embryogenesis and subsequent plant regeneration from nodal and internodal tissue-derived calli of *Bambusa edulis* was established. Shoots tips of a local accession (3-4 m in height) were used for multiple shoot production on Murashige and Skoog's medium (MS) supplemented with 0.1 mg/l thidiazuron (TDZ) and 3% (w/v) sucrose. Subsequent nodal and internodal tissues were utilized to obtain embryogenic calli on MS medium containing 2.0 mg/l kinetin (KN), 3.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.1% (v/v) coconut milk, and 6% (w/v) sucrose. Prolonged subculture (30 days each) of these embryogenic calli on the same medium resulted in embryo proliferation within 2 to 4 months. Optimal additives to the MS medium for embryo formation was determined to be 0.01 mg/l TDZ, 2.0 mg/l 2,4-D and 3% (w/v) sucrose. These embryos germinated on a MS-based medium containing TDZ at 0.1 mg/l, and developed into plantlets that were successfully transferred to soil. Flowers were on in vitro regenerants and potted plants, but no seed was produced. In vitro formed spikelets proliferated to produce more spikelets when subcultured on the MS basal medium containing TDZ. This spikelet-produce-spikelet culture has been maintained for over three years, and is being used to study reproductive physiology of this bamboo.

**THURSDAY, JULY 15, 1999**

**MOLECULAR ANALYSIS OF  
TREE GENOMES AND TREE GENETICS**

University of Oxford Natural History Museum,  
Lecture Hall

## SEMINAR 49

### THE USE OF RANDOM SEQUENCING IN THE ANALYSIS OF XYLEM CELL WALL FORMATION IN PINE

Isabel Allona

Unidad Docente Bioquímica, Departamento de Biotecnología , ETSI Montes  
Ciudad Universitaria, Spain

[iallona@etsi.montes.upm.es](mailto:iallona@etsi.montes.upm.es)

Secondary xylem formation is likely to involve some genes expressed rarely or not at all in herbaceous plants. To increase our understanding of xylem formation, and to provide material for comparative analysis of gymnosperms and angiosperm sequences, ESTs were obtained from immature xylem of loblolly pine. A total of 1097 single-pass sequences were obtained from cDNA libraries made from gravistimulated tissue from bent trees. About 10% of the recognised genes encode factors involved in cell wall formation. Sequences similar to cell wall proteins, most known lignin biosynthetic enzymes, and several enzymes of carbohydrate metabolism were found. A number of putative regulatory proteins also are represented. Two kind of studies have been performed. cDNAs coding for six novel cell wall associated proteins, as well as a homolog for a phytocyanin, have been identified and characterised from differentiating xylem of loblolly pine. Three cDNAs encode new putative loblolly pine arabinogalactan proteins based on their structural similarity to classical AGPs. One cDNA is related to proline-rich protein group and the other two to glycine-rich protein group and the mussel adhesive protein. Moreover, we have isolated and characterised, from pine xylem cDNA libraries, a set of clones representing a new subgroup of bHLH proteins. Basic helix-loop-helix (bHLH) proteins are an important class of regulatory proteins involved in control of cell fate in animals and yeast, and in the control of pigment synthesis in plants. Analysis of pine genomic DNA detects a single gene, suggesting that they are alternative splicing products derived from one gene.

## **SEMINAR 50**

### **POPLAR AND ARABIDOPSIS AS MODEL SYSTEMS FOR WOOD FORMATION**

Björn Sundberg

Dept. of Forest Genetics and Plant Physiology, SLU, Sweden

Bjorn.Sundberg@genfys.slu.se

<http://www.genfys.slu.se/>

Poplar has been extensively used in research directed towards understanding the molecular controls of wood formation. The utility of poplar as a model species for molecular research in woody plants has increased significantly with the recent Swedish initiative to produce large scale expressed sequence tag (EST) libraries from specific tissues. The database currently consists of 8,000 sequences from the wood-forming tissues and 5,500 sequences from mature leaves. Sequences are continuously added to the database and ongoing sequencing includes more tissue-specific libraries. The ESTs have been arranged in a microarray and these "wood chips" will initially be used to study global gene expression during different phases of xylem differentiation. Arabidopsis is an excellent model plant from a genetic point of view because of the large number of mutants available, extensive genetic maps and the large scale sequencing that is currently being undertaken by several groups around the world. Arabidopsis can also be used for studies on xylem formation because it undergoes extensive secondary growth in the root and hypocotyl when grown under appropriate conditions. The role of plant hormones in wood formation is being investigated using the EST database and the poplar and Arabidopsis as model systems. For example, ethylene-related genes, including genes responsible for ethylene synthesis, perception and signal transduction are well represented in the poplar EST library, and the expression of these genes in the poplar stem is investigated. In Arabidopsis, mutants affected in ethylene perception have altered xylem development. The significance of these findings and the utility of Arabidopsis and poplar to study wood formation will be discussed.

## **SEMINAR 51**

### **THE GENESIS PINE AND EUCALYPTUS EST PROJECTS.**

Timothy J. Strabala

Genesis Research and Development Corporation Ltd., New Zealand

t.strabala@genesis.co.nz

<http://www.biotech.org.nz/dir/genesis.htm>

EST sequencing on *P. radiata* and *E. grandis* has been underway at Genesis for three and a half years. In this time, we have constructed cDNA libraries from tissues including xylem, phloem, roots, reproductive organs, photosynthetic organs developing embryos and seedlings. From these libraries, we have obtained in excess of 100,000 sequences from each species. We estimate that we have obtained EST's of approximately 40% of the expressed genes for each species. Using a combination of polyT-primed and random-primed cDNA libraries, we have been able to decrease the numbers of contigs while simultaneously improving contig lengths. Analysis of expressed gene classes and comparison of the libraries to each other and the public databases will be discussed.



## **SEMINAR 52**

### **CONIFER COMPARATIVE GENOMICS PROJECT**

Garth R. Brown and David B. Neale

Institute of Forest Genetics, USDA Forest Service, Department of Environmental Horticulture, University of California Davis, USA

dbneale@ucdavis.edu  
<http://dendrome.ucdavis.edu/>

Comparative mapping has revealed the conservation of gene content and gene order over surprisingly long chromosomal tracts in several animal and angiosperm plant groups. We are investigating syntenic relationships in the genus *Pinus* to evaluate whether the more than 100 species can be viewed as a single genetic system, thereby allowing the transfer of genetic information between species. Loblolly pine, the most map-rich conifer with nearly 1000 genetic markers, serves as the reference to which other maps are compared. A set of 98 orthologous expressed sequence tag polymorphism (ESTP) markers has been developed and can be used for comparative mapping in pines and even other conifers (<http://dendrome.ucdavis.edu/Synteny>).

The ESTP markers are publicly available (<http://dendrome.ucdavis.edu/ESTPs>), as are DNA samples from the loblolly pine reference mapping populations (<http://dendrome.ucdavis.edu/Synteny/refmap.html>). An international collaboration, called the Conifer Comparative Genomics Project, has been initiated to construct comparative maps for all important pine and conifer species.

## SEMINAR 53

### **RAPD MARKERS LINKED TO A GENE FOR RESISTANCE TO PINE NEEDLE GALL MIDGE IN JAPANESE BLACK PINE (PINUS THUNBERGII)**

T. Kondo, K. Terada, E. Hayashi, N. Kuramoto, M. Okamura, and H. Kawasaki

Forest Tree Breeding Center, Japan (TK, EH, NK, MO, HK) and Tohoku Breeding Office, Forest Tree Breeding Center, Japan (KT)

konkon@nftbc.affrc.go.jp

Most major problem of tree breeding is necessary to take a long period because of long generation time. Allogamy and less genetic informations accumulated have also make it more difficult to improve. To cope with these problems, marker aided selection (MAS) has been expected as a new tool for breeding. As a major gene is most suitable as a marker for MAS, linkage of RAPD markers to a single dominant gene for resistance to pine needle gall midge was investigated in Japanese black pine (*Pinus thunbergii*). After screening 1160 primers using bulked segregant analysis, three primers produced three linked markers. The distances between the resistance gene and these markers were 5.1 cM, 6.7 cM and 13.6 cM. One marker was in coupling phase, and the other two markers were in repulsion phase to the resistance gene. Linkage map of the resistant tree was constructed using macrogametophytes. In linkage analysis, 98 out of 127 polymorphic markers were assigned to 17 linkage groups and 6 linked pairs. The total length of this map was 1469.8 cM with an average marker density of 15.6 cM. Genome length was estimated 2176 cM and this linkage map covered 67.5 % of the genome. Although the linked markers belonged to the same linkage group, no unique positions were found for two markers.

## **SEMINAR 54**

### **GENES POTENTIALLY INVOLVED IN THE SIGNAL TRANSDUCTION PATHWAY LEADING TO TREE DEFENSE AGAINST PATHOGENS: 14-3-3 CDNA CLONES ISOLATED IN WHITE SPRUCE AND HYBRID POPLAR**

G. Lapointe, M. D. Luckevich, and A. Seguin

Natural Resources Canada, Canadian Forest Service, Canada

seguin@cfl.forestry.ca  
<http://www.cfl.forestry.ca>

In the search for understanding the signal transduction pathway in the tree-pathogen interactions, we isolated few members of a gene family known as the 14-3-3. We obtained 5 clones from hybrid poplar and 3 from white spruce. Sequence comparisons show high homology between the fragments and higher than 75% homology with previously published sequences which seem involved in powdery mildew attacks. Treatments of trees with chitosan, jasmonates, or cold, or by wounding, caused either an increase or a slight down-regulation in 14-3-3 mRNA. Since jasmonates and chitosan are signal transducers of defense reactions in plants, our results suggest a possible role of 14-3-3 in pathogen defense response in trees. This role remains to be tested. In addition, PAL and CHS, two stress-related genes also involved in plant defense against pathogens, were used to verify their induction following treatment of trees with the previously cited elicitors. This is the first time 14-3-3s have been reported in tree species.

## SEMINAR 55

### QUANTITATIVE TRAIT LOCI (QTLs) FOR ECONOMICALLY IMPORTANT TRAITS IN *PINUS SYLVESTRIS*

E. Lerceteau, C. Plomion, and B. Andersson

Dept. of Forest Genetics and Plant Physiology, SLU-Umeå (EL), Laboratoire de Génétique et Amélioration des Arbres Forestiers, Station de Recherches Forestières-Pierroton (CP) and The Forestry Research Institute of Sweden (BA)

Estelle.Lerceteau@genfys.slu.se  
<http://linne.genfys.slu.se/pgen/staff/el.htm>

We used a two-way pseudo-testcross strategy for genetic mapping in *Pinus sylvestris* and for QTL detection of economically important traits included in the Swedish tree-breeding program. Based on 94 full-sib progenies of a cross between two plus-trees from Northern Sweden we generated two parental maps using AFLP (Amplified Fragment Length Polymorphism) markers. The female map comprised 99 markers into 14 linkage groups giving a size of 765.1 cM. On the male map 158 markers were assigned to 15 linkage groups for a total size of 1353 cM. The recombination frequency was found to be sex-dependent, being 29.2% higher in male than in female gametes. A difference in the QTL number detected for the male and female parents was observed. On the female map, ten QTLs were detected with a LOD value greater than 2.0, none for branch diameter and wood density. Three QTLs encoding tree height accounted for 25.8% of the total phenotypic variation. When the QTLs were taken independently, the percentages of phenotypic variance ranged from 9.3% to 23.7%. The highest value was observed for frost tolerance, an important trait in Northern Sweden for which a major gene seemed to be involved. A cluster of QTLs for tree height, trunk diameter and volume was located on one linkage group. On the male map, three QTLs for trunk diameter and volume were detected. The efficiency of QTL detection is discussed based on the complexity of the traits, their variability and the strategy used.

## **SEMINAR 56**

### **GENETICS OF THE MARITIME PINE PROTEOME**

C. Plomion, P. Costa, C. Dubos, J-M. Frigerio, C. Pionneau

INRA - Forestry Department, France

plomion@pierroton.inra.fr

<http://www.pierroton.inra.fr/genetics/>

The concept of proteome has emerged recently as a consequence of question raised in the context of genome and post-genome project. The genetic variation of the maritime pine proteome, and its application for addressing genetical and physiological questions will be presented. A number of proteins showing mendelian inheritance in a segregating progeny, were localized on a DNA-based genetic map, providing interesting markers to map the expressed genome. Such markers are physiologically relevant in that they reveal loci whose transcripts are translated in the organ analyzed. The variability of xylem and needles protein expression, was studied in the context of gravitational and drought stress responses, respectively. In respect to the first point, compression wood-responsive proteins were identified and characterized. In respect to the second point, protein profiles of a number of seedlings were obtained in two contrasted conditions for water availability. The loci controlling protein accumulation were mapped on the genome. Such PQL (protein quantity loci) were localized for a number of proteins and their position compared between the stressed on non-stressed environments. Beyond the interest for regulatory genetics, the PQL methodology can provide additional tool for the difficult task of identifying QTLs in the context of the candidate gene approach. Also, in both cases (gravistimulated and drought-responsive proteins), clustered correlation analysis allowed the identification of groups of proteins with similar expression profiles. Finally, unambiguous identification of 50 needle and xylem proteins excised from the gels was obtained by internal micro-sequencing and electrospray ionisation mass spectrometry, providing the basis of a proteomics database.

## SEMINAR 57

### GENETIC MAPPING OF POPLAR

V. Storme, J. Zhang, M.-T. Cervera, M. Steenackers, B. Ivens, S. Neyrinck, K. Schamp, B. Michiels, H. Zhang, M. Van Montagu and W. Boerjan

VIB, University of Gent, Belgium (VS, JZ, MTC, BI, MVM, WB), Institute of Forestry and Game Management, Geraardsbergen, Belgium (MS, SN, KS, BM) and Texas AM, Houston, Texas, USA (HZ)

vesto@gengenp.rug.ac.be  
<http://www.plantgenetics.rug.ac.be>

Poplar is one of the most planted tree species in Europe. Its wood is used for a variety of end products such as pallets, veneer and pulp. Due to the long generation times of trees, the genetic improvement of trees by conventional breeding lags far behind that of annual plants. The possibility to generate genetic maps allows to unravel the genetics of particular traits and to identify the genes behind these traits. This new information can be of great value to improve classical breeding programs and opens possibilities to further improve elite genotypes by genetic engineering. Three genetic maps of poplar (*P. deltoides*, *P. trichocarpa* and *P. nigra*) were constructed using the pseudo-testcross strategy in combination with AFLP. The maps were generated from 2 controlled crosses sharing the same female parent (*P. deltoides* 'S9-2' x *P. nigra* 'Ghoy' and *P. deltoides* 'S9-2' x *P. trichocarpa* 'V24'). Microsatellite markers (SSR), derived from the Poplar Molecular Genetics Co-operative were used to align the three genome maps. Bulk segregant analysis was used to fine-map the *Melampsora larici-populina* resistance gene (*MER*) that is present as a single dominant gene in the *P. deltoides* parent and that confers resistance against race E2. *M. larici-populina* is one of the most damaging fungal pathogens for poplar in Europe. To clone this resistance gene, a binary BAC library has been constructed from the resistant *P. deltoides* genotype. The flanking markers are being used to identify BAC clones that contain the *MER* gene by filter hybridisation.

## SEMINAR 58

### LINKING PHYSIOLOGICAL TRAITS TO MOLECULAR MARKERS: PUTATIVE QTL FOR LEAF GROWTH IN POPLAR

G. Taylor, S.Bunn, R. Ferris and H.D. Bradshaw

School of Biological Sciences, University of Southampton, UK and College of Forest Resources, University of Washington, USA

g.taylor@soton.ac.uk  
<http://www.soton.ac.uk/~gt1/>

Three decades of research on poplar have illustrated the links between physiological traits and the productivity of particular genotypes. Several characteristics may be considered as 'key' traits for yield and this paper will focus on one such trait - the development of leaf area. A plethora of information exists to support the notion that genotypes which produce large leaves, have rapid stemwood production (VanVolkenburgh and Taylor, 1996). Evidence to confirm this for UK-grown poplars in short rotation coppice will be presented. Leaf growth is the result of the combined effects of leaf cell production and leaf cell expansion. Our research shows a clear relationship between leaf area and leaf cell production, with no such relationship between cell size and leaf area, perhaps reflecting the biophysical nature of the cell expansion process (Bunn *et al.*, 1999). Using a mapping population of poplar (Bradshaw, 1996) we have identified putative QTL for leaf cell expansion and production in both ambient and elevated carbon dioxide.

Bradshaw HD (1996). Molecular genetics of *Populus*. In: Biology of *Populus* Ed. by RF Stettler, HD Bradshaw Jr., PE Heilman, TM Hinckley. NRC Research Press, pp. 183-197. ISBN: 0-660-16506-6.

Bunn S, Herbert C and Taylor G (1999). Physiological basis of enhanced yield of poplar (*Populus trichocarpa* X *P. deltoides*) grown as short rotation coppice. (submitted)

VanVolkenburgh E and Taylor G (1996). Leaf growth Physiology. In: Biology of *Populus*. Ed. by RF Stettler, HD Bradshaw Jr., PE Heilman, TM Hinckley. NRC Research Press, pp. 283-300. ISBN: 0-660-16506-6.

## SEMINAR 59

### QTL FOR GROWTH IN EUCALYPTUS NITENS

G.F. Moran, L. Emebiri, K. Groom, J. Owen, M. Byrne, and E. Williams

CSIRO Forestry and Forest Products, Australia

[gavin.moran@ffp.csiro.au](mailto:gavin.moran@ffp.csiro.au)

<http://www.ffp.csiro.au/tigr/molecular.html>

Initial work on QTL for biomass in *Eucalyptus nitens* centred on seedling growth parameters including height and leaf area at three months of age in a glasshouse study. A number of QTL were located on linkage groups including three QTL affecting height and two QTL affecting leaf area. Both of the QTL for leaf area were in same linkage regions as QTL that affected height. The pedigrees were planted in a field trial and assessed for growth and other traits annually. Two experiments were planted within the field trial with seedlings from the glasshouse trial planted in experiment one and additional progeny from the pedigrees planted in experiment two. From experiment one expression of QTL for growth were found to vary over time with some QTL stably expressed from 3 month old seedlings to four year old trees. Two of the major QTL expressed in seedlings were not evident after two years of age. Some were expressed from two years of age onwards. In addition some QTL showed strong expression from early field growth but were not in evidence at age four and other QTL showed the opposite pattern. All these results are really not surprising given the often poor correlations of early glasshouse performance and later age performance. Field growth data from experiment two was used to test for validation of the QTL for stem growth. All markers showing significant associations with either height or diameter were assessed in progeny of pedigrees in experiment two. The implications of results from this validation process will be discussed.



## **SEMINAR 60**

### **QTL DETECTION IN A FACTORIAL MATING DESIGN: ANALYSIS OF WOOD MICRODENSITOMETRY CHARACTERS IN LARCH**

D. Prat, A. Arcade, P. Faivre Rampant, L.E. Pâques

Unite Amelioration, Genetique et Physiologie forestieres, INRA Centre d'Orleans, France

prat@orleans.inra.fr

The use of wood quality traits as selection criteria in forest breeding is hampered by long delays before evaluation. The feasibility of early selection using genetic markers cosegregating with the trait of interest, or involved in its control, could change this situation. Forest tree species are characterised by the lack of three-generation pedigrees in most species and by high level of polymorphisms. Consequently, F1 generation can be used for genetic mapping and QTL detection. Using F1 progeny, a QTL can be detected if it is in an heterozygous state in one parent at least. The analysis of a single family decreases the probability to detect QTL. On the other hand, the use of several families increases this possibility, providing that the genotyping activity is kept at a convenient level. We have developed a method of QTL detection in a factorial mating design. It is based on the relationships between parent genotypes and family performance. Further validation of putative QTLs were done within a set of families segregating for the associated markers. We have applied this method to a 12 x 12 factorial mating design between European and Japanese larches. Wood quality traits were assessed by microdensitometry on 16-year-old trees. A genetic map based on AFLP, RAPD and ISSR markers is available. Several genomic regions are involved in the control of traits. Some of these regions were also involved in the same traits by the analysis of a single family. Additional putative QTLs were detected and should be confirmed.

**THURSDAY, JULY 15, 1999**

**THE APPLICATION OF MOLECULAR  
GENETICS AND BIOTECHNOLOGY  
IN CONSERVATION  
AND WITH NOVEL SPECIES**

University of Oxford Natural History Museum,  
Lecture Hall

## SEMINAR 61

### **BIOTECHNOLOGY, GENOMICS AND BIODIVERSITY: HOW CAN WE PUT THEM ALL TOGETHER IN NORWAY SPRUCE?**

M. Morgante, F. Cattonaro, S. degli Ivanissevich, I. Jurman, G.P. Paglia, and I. Scotti

Ag Biotechnology, Genomics, El DuPont de Nemours and Co. (Inc.), USA, (MM) and Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Universita' di Udine, Italy (MM, FC, SDI, IJ, GPP, IS)

michele.morgante@usa.dupont.com

Plant biotechnology continues to benefit from the insights being developed in the Human Genome project. The proliferation of mapping, genotyping, sequencing, profiling and diagnostic methodologies has rapidly expanded the analytical tools available to plant scientists. A whole new discipline has developed, called genomics, which comprises structural genomics (the discovery, sequencing and mapping of genes and genomes) and functional genomics (the understanding of the function of each gene and of the means by which they are controlled). The impact and the potential applications of genomics in the plant area are even greater than in the human area because they are not only limited to understanding the mechanisms of diseases and how to prevent them but encompass the understanding and manipulating of both the basic and secondary metabolism. This may allow us in the future to obtain most of our food, fuel, fiber, chemicals and even pharmaceuticals from crops and trees. Together with the identification and functional analysis of all genes in one or a few species (vertical sampling), a thorough investigation of allelic and gene diversity within the plant kingdom (horizontal sampling) is going to be of paramount importance for plant and food improvement. Even though new alleles and genes can be created in vitro, the existing diversity both in terms of different alleles

## SEMINAR 62

### DISTRIBUTION OF THE HAPLOTYPIC DIVERSITY IN CONIFER AND BROADLEAVED SPECIES AS REVEALED BY CHLOROPLAST MICROSATELLITES

G.G. Vendramin, M. Anzidei, S. Fineschi, A. Madaghiele, R.J. Petit, B. Ziegenhagen

Istituto Miglioramento Genetico Piante Forestali, CNR, Firenze, Italy (G.G.V., M.A., A.M)  
Istituto per l'Agroselvicoltura, CNR, Porano (TR), Italy (S.F.) Laboratoire de Genetique et  
Amelioration des Arbres Forestiers (INRA), Gazinet, France (R.J.P.) Institute for Forest  
Genetics and forest Tree Breeding (BFH), Grossahansdorf, Germany (B.Z.)

vendramin@imgpf.fi.cnr.it

During the last years great interest has been dedicated to the analysis of the distribution of haplotypic diversity in both conifers and broadleaved forest tree species using uniparentally inherited markers. In particular, the analysis of chloroplast microsatellites (simple sequence repeats) resulted to be extremely informative because of their high degree of polymorphism and of the universality which allowed the use of these markers in a wide range of different species. Primers for the amplification of mononucleotide stretches identified in the chloroplast genome of *Pinus thunbergii* and *Nicotiane tabacum* were used to screen the variation of these simple sequence repeats and to estimate diversity in conifers and broadleaved species, respectively. An extremely high level of diversity within and among populations were detected in all conifers analysed so far: the analysis of the distribution of the haplotypic diversity provided important information about the possible recolonisation processes which occurred in the post-glacial period starting from the refugial areas. Evidences of possible hybridisation among different species were also detected. Polymorphism was also observed in broadleaved species. The distribution of the diversity within and among populations varies evidently in the two taxonomic groups, revealing a much higher genetic differentiation among populations in broadleaved species than in conifers, due to the different mode of transmission of the chloroplast genome (paternal inheritance in conifers and maternal in broadleaved species). Examples of the application of these markers in *Pinus*, *Picea*, *Abies*, *Fagus*, *Fraxinus*, *Tilia* and *Crataegus* species will be presented and discussed.

## SEMINAR 63

### MICROSATELLITE ANALYSIS OF SEEDLOT SAMPLES FROM PEDUNCULATE OAK: INFERENCE OF THE SEED PARENTS AND POLLEN DONORS FROM THE OFFSPRING

C. Lexer, B. Heinze, S. Gerber, H. Steinkellner, B. Ziegenhagen, A. Kremer, J. Gloessl

Centre of Applied Genetics, Agricultural University of Vienna, Austria (CL, HS, JG),  
Federal Forest Research Centre Vienna, Austria (BH), INRA Pierroton (SG, AK), Institute  
of Forest Genetics Grosshansdorf, Austria (BZ)

clexer@edv2.boku.ac.at

[http://hal.boku.ac.at/research/en\\_search\\_unit.show\\_unit?unit\\_id\\_in=6](http://hal.boku.ac.at/research/en_search_unit.show_unit?unit_id_in=6)

We have tested microsatellite analysis to monitor within-stand genetic diversity in seedlot samples from pedunculate oak (*Quercus robur*). We focussed on two key criteria of within-stand diversity: The number of mother trees (seed parents) included in the seed harvests, and the number of father trees (pollen donors) contributing to the open pollinated families. Our results show that nuclear microsatellite loci are suitable to detect seed contaminations and to infer the genotypes of the seed parents directly from genotype data of extremely small anonymous acorn samples (5 to 8 acorns per mother tree), given that the samples from each putative mother tree have been supplied separately. Subsequently the number of different mother trees for such anonymous seedlot samples can be inferred by comparing the reconstructed maternal genotypes and by calculating  $F_{st}$  pairwise between the families. Furthermore we have tested an approach to infer the number of different pollen donors directly from open pollinated families by analysing haplotype arrays of closely linked microsatellite markers transmitted from the fathers to the progeny. We selected 3 closely linked microsatellites from a previously constructed genetic map of *Quercus robur*, then we used simulated data of these linked markers segregating in open pollinated families to test the approach in detail. The procedures presented here are designed for small sample sizes and for situations where no genotype information is available for the parent population. Therefore they are potentially useful for monitoring commercial seedlot samples from oak.

## SEMINAR 64

### MOLECULAR GENETIC INVESTIGATIONS IN WILD BIRD CHERRY AND SWEET CHERRY CULTIVARS (*PRUNUS AVIUM* L.) IN CENTRAL EUROPE

B. Heinze

Institute of Forest Genetics, Federal Forest Research Centre, Austria

Berthold.Heinze@fbva.bmlf.gv.at

<http://www.fbva.bmlf.gv.at>

The wild cherry (*Prunus avium* L.) is considered a native species of Central European hardwoods, whereas the cultivated sweet cherry of the same botanical classification was introduced here in Roman times. Differences in growth performance may exist between these two, therefore it is essential to certify forest reproductive material in the seed and nursery stages. We are investigating chloroplast and nuclear DNA markers for this purpose. Three chloroplast haplotypes have been identified by PCR-RFLP in the large single-copy chloroplast DNA region to date. The most common one among these is shared by the majority of wild cherry samples collected, and a number of ancient cultivars. Two more haplotypes showing a number of mutations relative to the previous type are found in two common cultivars, but also in several cherry trees in open agricultural land, previously considered as wild cherries. To investigate whether the two latter haplotypes have been introduced from other geographic regions (i.e., glacial refugia) of the species distribution range, or together with fruit cultivars in historic times, we have initiated analysis of plant material mainly from south-eastern Europe.

## SEMINAR 65

### THE DYNAMICS OF MATING IN A FRAGMENTED POPULATION OF *SWIETENIA HUMILIS* (ZUCC.) USING SSRs AS A MARKER SYSTEM

D. Boshier, G. White, W. Powell

Oxford Forestry Institute, University of Oxford, UK (DB). Scottish Crop Research Institute, UK (GW, WP). Current Address: Boyce Thompson Institute, USA (GW). Current Address: DuPont, Delaware Technology Park, USA (WP)

david.boshier@plants.ox.ac.uk  
<http://www.plants.ox.ac.uk/of/>

The effects of deforestation and forest fragmentation on the population dynamics and consequent genetic conservation value of forest remnants and trees on farms, is a topic of debate. The use of direct pollen flow measures to inform about the long term genetic viability of fragmented populations has been limited by; a) the power of genetic markers to discriminate pollen donors, and b) the initial sampling strategy and degree of fragmentation. The talk describes the use of simple sequence repeats (SSRs) as a hypervariable marker system to study a highly fragmented population of *Swietenia humilis* (Zucc.) in Honduras. *S. humilis* is one of the three timber species commonly known as mahogany. Over much of its native range in the dry forests of the Central American and Mexican Pacific watershed, its populations have become reduced and highly fragmented, with the species currently listed in Appendix II of the Convention on International Trade in Endangered Species (CITES). Progeny arrays from selected trees within each fragment were genotyped at 4 SSR loci and paternity exclusion analysis performed. A large proportion of pollen flow was from outside of each fragment indicating an extensive network of gene exchange over the spatial scale under study. Different patterns of pollen flow were evident both within and between the fragments, influenced by fragment size and flowering phenology. The genetic impacts of fragmentation are undoubtedly complex and will vary between species, but for some tree species pollination appears to occur over considerably greater distances and more frequently than has previously been considered.

## SEMINAR 66

### RAPD DETECTION OF NARROW-LEAF PHENOTYPES IN THE REGENERATED PLANTLETS OF HYBRID POPLAR

K. Ishii, R. Thakur

Forestry and Forest Products Research Institute, Japan

katsuaki@ffpri.affrc.go.jp  
<http://www.ffpri.affrc.go.jp/>

Narrow-leaf phenotypes were identified among a population of hybrid poplar (*Populus sieboldii x grandidentata*) regenerated through *in vitro* culture. Variants appeared alike among themselves in appearance but differed significantly in appearance and size from the donor plant. Morphological studies revealed that phenotypic variants had higher number of branches and had much smaller, narrower and thicker leaves than the parent tree. To elucidate the basis of these phenotypic variation, RAPD analysis was performed on variations as well as the normal plants. Among the 120 decamer primers of Operon Technology, Calif.; only four primers viz., OPG 12, OPQ 04, OPF 20 and OPT 02 differentiated normal plants from the variants. Only one primer, OPB 04, differentiated one mutant from the normal plant and another mutant with an additional band at 350 bp. Out of 386 reproducible markers from 120 primers obtained, only 4 correlated with narrow-leaf phenotypes. One band specific to narrow-leaf variant was sequenced and the homology analysis using the BLAST data base was done.



## SEMINAR 67

### APPLICATION OF CRYOPRESERVATION TO A CLONAL EUCALYPTUS IMPROVEMENT PROGRAMME

D Blakesley and R Kiernan

Horticulture Research International, UK (DB), Shell Forestry, Horticulture Research International, UK (RK)

David.Blakesley@hri.ac.uk  
<http://www.hri.ac.uk/>

Shell Forestry are using genetic modification strategies for the improvement of elite clones of a *Eucalyptus grandis* hybrid. Following genetic transformation, *in vitro* shoot cultures must be stored during field evaluation of multiple transgenic lines. The length of storage would depend on the trait being assessed, but could be 5 years or more in the case of altered wood quality. Furthermore, beyond this assessment, storage of elite transgenic lines would be required if the plant material were to be available to customers in the future. These requirements create a need for low cost and safe methods of storage, which also reduce the risk of somoclonal variation during evaluation. Cryopreservation has been identified as the method with most potential for long term storage to support the GM programme. Consequently, a pilot study was undertaken to develop a cryopreservation protocol for elite *Eucalyptus* genotypes. An encapsulation/dehydration protocol has achieved more than 50% survival of shoots of Genotype I. Several other genotypes have been tested, and promising results obtained with Genotype II, which gave slightly lower survival rates after freezing. In contrast, two other genotypes (III and IV) gave very poor survival after freezing, of just 3%. A non-encapsulation protocol has achieved up to 38% survival of shoots of Genotype I. Although survival did not match that obtained with encapsulation protocols, the non-encapsulation protocols are much simpler than those requiring encapsulation, and do not require sophisticated slow cooling equipment.

## SEMINAR 68

### BIOTECHNOLOGY OF POPULUS EUPHRATICA IN INDIAN SEMI-ARID TRANS GANGETIC PLAINS

A. Sharma

Tata Energy Research Institute, India.

Extremely harsh climatic conditions, mounting biotic pressure and degraded soils in semi-arid and arid regions not only restrict the choice of species but also hinder the efforts to counter ever expanding fuel and fodder scarcity. Poplars have been widely accepted for mass plantations and agroforestry systems in the Indian subcontinent. *P. euphratica*, *Oliv.* has been acclaimed for its better survival and biomass production on wide range of sites in dry arid areas of Mangolia, China, Pakistan, Iraq, and Iran. It was introduced at the Biomass Research Centre of Tata Energy Research Institute, Gurgaon (Haryana) in 1987 to explore its potential in mitigating the fuel and fodder crisis in semi-arid and arid regions of India. The species is known for withstanding fluctuating water table. The introductory plantation of *P. euphratica*, by root suckers, sets, stem and branch cuttings, was raised on a site which remains water-logged for more than three months a years. The plantations were appraised for their survival as well as growth. Later the growth performance was compared with adjoining other *P. deltoides* clones being tested at the research centre. The results emphasis the adaptability of the species to the edaphic and climatic conditions. Also the plantations raised from the root suckers were found to performance better, both in terms of diameter and height growth. Almost seven hectare of water logged site was revegetated in three years, by planting the root suckers. It was further observed that the species regenerate through profuse suckering.

# POSTERS

## POSTER 1

### **KINETICS OF ETHYLENE BIOSYNTHESIS AND ITS EFFECTS DURING MATURATION OF WHITE SPRUCE SOMATIC EMBRYOS.**

A. El Meskaoui and F. Tremblay

Centre de Recherche en Biologie Forestière, Université Laval, Canada

abdelmal@rsvs.ulaval.ca

The objective of the current investigation was to study the role of ethylene on the maturation of white spruce somatic embryo. This was done by examining the effect of 1-aminocyclopropane-1-carboxylic acid (ACC), silver nitrate ( $\text{AgNO}_3$ ), aminooxyamino acid (AOA), and by pure ethylene enrichment on maturation of somatic embryo. Both treatments with AOA, a potent inhibitor of ACC synthase that plays a role in ACC synthesis, or with  $\text{AgNO}_3$ , an inhibitor of ethylene action, produced global opposite effects to those of ACC and ethylene, gives support to the conclusion that a reduction of ethylene is beneficial to maturation of somatic embryo. This is further substantiated by the fact that inhibitor effects of AOA are partially reversed by adding ethylene. The possible effect of the interaction between ethylene and polyamines metabolisms on somatic embryo development was also discussed.

## POSTER 2

### IN VITRO PROPAGATION OF MARITIME PINE FOR ADDED-VALUE FORESTRY

S. Gonçalves, S. Tereso, L. Marum, C. Miguel and M. M. Oliveira

Lab Pinus - Instituto de Biologia experimental e Tecnológica, Portugal

In Portugal the forest and its associated industry, represent a major economical sector that was considered to be one of the clusters that could develop competitive advantages for the country. Pine forest in Portugal almost completely consists of *Pinus pinaster* (maritime pine). However, industrial activity in Portugal may soon face serious problems due to insufficient wood production. In an integrated program for the genetic improvement of maritime pine, the development of *in vitro* propagation techniques for easy clonal propagation of superior genotypes need to be developed. The main goal of this project is the establishment of several methods for *in vitro* propagation of maritime pine (*Pinus pinaster*). Three main research areas will be investigated for clonal propagation of superior genotypes:

1. definition of protocols for *in vitro* propagation by axillary branching,
2. definition of protocols for production of somatic embryos derived from mature and/or immature seeds,
3. methods for adventitious regeneration from embryos isolated from mature seeds

We have accomplished the induction of adventitious buds from cotyledons of mature seeds and experiments are being conducted to optimize the elongation phase. From 2 year-old plants we have obtained axillary branching with a weekly-based pulverization of cytokinin. These newly formed shoots were surface sterilized and transferred to *in vitro* culture where are being used in experiments for optimal propagation conditions. The induction of somatic embryogenesis is being performed using immature embryos collected from superior genotypes. The optimal stage to achieve response is being tested, as well as different culture media and conditions.

### POSTER 3

#### **STUDIES ON FACTORS AFFECTING PROTOPLAST ISOLATION AND CULTURE OF ULMUS MINOR MILL.**

P. Conde, C. Santos, A. Sousa and G. Caldeira

Cell Biology Center, Department of Biology, University of Aveiro, Portugal

pconde@bio.ua.pt  
<http://www.ua.pt>

Elm is a woody plant performing an important role in Portuguese landscape. Unfortunately Dutch elm disease is seriously affecting this species, like many elm species across the world. We are studying this disease and assaying for resistant plants *in vitro* which implies the development of techniques of *in vitro* elm regeneration. We report here the isolation of viable protoplasts from callus and *in vitro* and *ex vitro* leaves of ULMUS MINOR. Several factors were studied in protoplast production: enzyme (Cellulase Onozuka RS and R10, Driselase Fluka, Macerozyme Onozuka R10 and Pectinase Fluka), tissue source (callus, *in vitro* and *ex vitro* leaves) and leaf position in shoot. Leaves used here were obtained from greenhouse plants (22°C+2, photoperiod 16/8h) and from *in vitro* plants regenerated on DKW medium. Callus was obtained from leaves on MS medium. Protoplast isolation was performed in CPW medium with 13% mannitol and after 16 h of digestion at 40 rpm, protoplasts were purified using CPW medium with 21% sucrose. The best yields were obtained in the combinations presenting Onozuka RS. Onozuka R10 seems to be ineffective in protoplast isolation from *ex vitro* leaves. This enzyme although effective with *in vitro* leaves, often leads to low yields. *Ex vitro* leaves also presented usually lower yields than the other ones. Several enzyme combinations gave high protoplast productions (around 1000000-10000000 protoplasts/g of tissue). Purified protoplasts were cultivated in KM8p and SH media. Colony and callus formation is being followed daily.

## POSTER 4

### INFLUENCE OF EXPLANT SOURCE, MEDIA AND ENVIRONMENTAL CONDITIONS ON CULTURE INITIATION AND CALLUS PRODUCTION IN QUERCUS SUBER AND ROBUR.

H. Azevedo, C. Santos, R. Campos and G. Caldeira

Cell Biology Center, Department of Biology, University of Aveiro, Portugal

csantos@bio.ua.pt  
<http://www.ua.pt>

Portugal is one of the greatest cork producers in the world, and *Quercus* production has an important significance in Portuguese economy. Cork oak trees used nowadays are old and affected with several diseases. We are trying to select cork oaks with high quality and to regenerate them by *in vitro* culture. Also *Quercus robur* (oak) is an important source of high quality wood. Several factors were assayed on culture initiation of greenhouse shoots (22°C +2, photoperiod 16/8h) cork oak and oak plant seedlings. Buds were sterilised and placed on modified Heller and Gresshoff and Doy media. Different combinations of growth regulators were assayed (IAA, NAA and BAP). Axillary buds were identified by their position in the shoot. Cultures were placed in different light conditions and culture initiation was followed daily. In cork oak, basal buds did not survive and showed high levels of infection. On the other hand, buds from the basal-medium position developed callus tissue not developing new shoots while the apical buds developed mainly new shoots. Light seems to play an important role in shoot/callus regeneration in oaks. Under some conditions we observe the development of lots of small shoots with a null development of the internodes. Structural changes in regenerated tissues and organs are being followed by microscopy.

## POSTER 5

### DEVELOPMENT OF SOMATIC EMBRYOGENESIS IN *PINUS ELLIOTTII* X *P. CARIBAEA*

O.S.P Dickens, A-M Botha and C.H. Bornman

Dept of Genetics, Forestry and Agricultural

Institute, University of Pretoria, Pretoria, 0002, South Africa

ambothao@postino.up.ac.za

Regeneration of plants is an important factor in any somatic embryogenesis programme. Also important is an understanding of the functional development of the somatic embryo, as well as the roles of the chemical and physical environments during initiation, which in the conifer almost invariably is based on the immature zygotic embryo. It has been reported in *Larix* that somatic embryos may be initiated from cells of the secondary suspensor, and in *Picea mariana* initiation has been observed from cells of the stomatal complex without the embryogenic tissue apparently having undergone the characteristic stage of embryo-suspensor-mass (ESM). We have excised zygotic embryos of a pine hybrid (*Pinus elliotii* x *P. caribaea* var. *hondurensis*) and cultured them separately from the megagametophyte, and in some cases also the suspensor, on a Murashige and Skoog medium lacking ammonium salts but containing glutamine and varying concentrations of auxin and cytokinin. Samples of the cultured tissues at various stages of development were fixed in glutaraldehyde and embedded in glycolmethacrylate. Thin sections were cut with a glass knife and stained with either toluidine blue and/or periodic acid Schiff's reagent for histological examination. So far, our observations show that the ESM derives from dedifferentiating cells of either the cotyledons or the apical part of the hypocotyl, but not from cells of the root cap, root apex or suspensor.



## POSTER 6

### STUDIES ON THE IN VITRO ROOTING ABILITY OF COLD-TOLERANT HYBRIDS OF EUCALYPTUS GRANDIS X NITENS AND ASSESSMENT OF ROOT QUALITY

M.E.O Mokotedi., M.P. Watt., N.W. Pammenter., and F. Blakeway

University of Natal,Durban (M.E.O., M.P.W., and N.W.P) and Mondi Forests (F.B)

mokotedi@biology.und.ac.za

<http://www.nu.za.biology/default.asp>

The South African Forestry industry needs to extend its plantation areas to continue to meet the demand for *Eucalyptus* products. Clones of hybrids of fast growing *Eucalyptus grandis* and cold-tolerant *E.nitens* (GN hybrids) have been identified as being highly desirable for this purpose. However, their production has been hampered by the poor rooting ability of the clones, both from macrocuttings and micropropagation. This study aimed at investigating factors influencing *in vitro* rooting, thereby establishing a culture protocol for high yielding rooted plantlets of two clones, GN121 and GN107. The highest rooting efficiencies in clones GN121 (75 - 80%) and GN107 (65%) were achieved in  $\pi$  MS modified by increasing calcium and magnesium levels to those of  $\leq$  MS. Other medium components were 0.1 mg.l<sup>-1</sup> IBA, 0.1 mg.l<sup>-1</sup> biotin, mg.l<sup>-1</sup> calcium pantothenate, 15.0 g.l<sup>-1</sup> sucrose and 4.0 g.l<sup>-1</sup> Gelrite. Best culture conditions were an initial 72-hour dark incubation followed by a 16-hour photoperiod at a PPFD of 37.0  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> and 23°C day/21°C night for seven days, after which the PPFD was increased to 66.25  $\mu$ mol.m<sup>-2</sup>.s<sup>-1</sup> at 27°C day/21°C night for 18 days. As the commercial application of any micropropagation protocol for tree species depends on the quality of the roots produced, we report also on ongoing investigations aimed at assessing and comparing roots produced *in vitro* and from macrocuttings, with respect to anatomy and physiological characteristics (e.g. hydraulic conductivity).

## **POSTER 7**

### **INTER AND INTRA FAMILY EFFECTS IN SOMATIC EMBRYOGENESIS OF SCOTS PINE**

C. Ramirez-Serrano, P. Bozhob, I. Ekberg, and S. von Arnold

Department of Forest Genetics, Swedish University of Agricultural Sciences

Carlos.Ramirez.Serrano@sgen.slu.se  
<http://www.sgen.slu.se/Skogsgenetiks>

The use of somatic embryogenesis in conifer improvement programmes is usually limited for the low range of genotypes that are able to produce sufficient amounts of high quality somatic embryos. The aim of our project was to assess the seed family and individual genotype effects on scots pine somatic embryogenesis, and to verify a possibility to produce high-quality somatic embryos from a large number of genotypes. As many as 36 half sib families were selected for this analysis. Embryogenic cell lines were established from 21 families with a frequency varying from 0.5 to 7%. Mature somatic embryos with no morphological aberrations were produced in 70 out of 82 tested cell lines belonging to 20 families.

## POSTER 8

### SELECTION PROTOCOL FOR PINUS RADIATA AND PICEA ABIES BASED ON THE APHIV RESISTANCE GENE

J. Charity, A. Wagner, J. Moody, L.J. Grace and C. Walter

Genetic Engineering for Future Forests, Forest Research, New Zealand

julia.charity@forestresearch.co.nz  
<http://www.forestresearch.cri.nz>

Genetic engineering is a very attractive technique for basic and applied forestry science, since it allows the investigator to modify a specific trait of the target organism, without altering the genetic background of the selected clone. Furthermore, this technology has the potential to add traits to the target organism which are not readily accessible to conventional breeding techniques. These are some of the reasons for the enormous effort in recent years directed at the establishment of transformation protocols for forest trees of economic importance, such as *Populus tremula*, *Eucalyptus grandis* and conifers such as *Larix decidua*, *Picea abies* or *Pinus sylvestris*. Recently we described a transformation system for *Pinus radiata* and other conifers based on a Biolistic® transformation protocol using NPTII as a selective marker. Selection genes such as the APHIV gene from *E. coli*, which confer resistance to the aminoglycoside antibiotic hygromycin B, have been successfully used in the past to transform a number of angiosperm species such as tobacco, *Arabidopsis*, *Brassica*, rice, maize and wheat. Recently the first stable transformation of a conifer species (black spruce) using a hygromycin B based selection protocol was reported. We were able to show that hygromycin B based selection is also applicable to conifers such as *Pinus radiata* and *Picea abies*. The establishment of a selection system based on the APHIV resistance marker using hygromycin B as the selective agent will widen our potential to genetically engineer different conifer species.

## POSTER 9

### CONIFER GENETIC ENGINEERING FOR HERBICIDE RESISTANCE: TRANSGENIC PLANTS OF PINUS RADIATA AND PICEA ABIES ARE RESISTANT TO THE HERBICIDE PHOSPHINOTHRICIN

Charity, C Walter, S. Bishop-Hurley, J Ray, J Zabkiewicz and L Grace

Forest Research, New Zealand

[julia.charity@forestresearch.co.nz](mailto:julia.charity@forestresearch.co.nz)

<http://www.forestresearch.cri.nz>

Herbicide resistance is one of the most important targets for genetic engineering in commercial forestry, mainly because one difficult weed control problem in the future, will be intra-species competition. While herbicide resistant agricultural plants are in commercial use, no data has been available for conifers. We have cloned the BAR gene for resistance against the herbicide phosphinothricin (Buster, Basta) and introduced it into *Picea abies* and *Pinus radiata* using Biolistics. Several transgenic lines (transclones) were selected, and plants were regenerated. The expression level of the bar gene was found to be highly variable between transclones. Plants from one transclone of *P. radiata* and 5 transclones of *P. abies*, plus control plants were spray-tested with a commercial herbicide formulation, including a surfactant. Concentrations representing 0.5, 1 and 2 Kg herbicide per hectare were used. Earlier spray testing of gorse, a major New Zealand forest pest, had confirmed the optimal operational concentration for the herbicide. All but one *P. abies* transclone survived the spray testing, whereas controls were dead within four weeks. The resistance level displayed by transgenic *P. abies* plants reflected the expression level of the bar gene and the lowest expressing transclone did not survive the spray test. Transgenic *P. radiata* however displayed full resistance even at a very low expression level. The results demonstrate that herbicide resistance may be a valid concept in commercial forestry. Herbicide resistant conifers will be field trialed in New Zealand and experiments to engineer resistance against sulfonylurea herbicides and glyphosate, are in progress.

## POSTER 10

### AGROBACTERIUM-MEDIATED TRANSFORMATION OF PINUS RADIATA COTYLEDONS.

J.A. Charity, L. Holland, S. Donaldson and C. Walter.

Genetic Engineering for Future Forests, Forest Research, New Zealand

[julia.charity@forestresearch.co.nz](mailto:julia.charity@forestresearch.co.nz)

<http://www.forestresearch.cri.nz>

A protocol using *Agrobacterium tumefaciens* to transfer the *uidA* reporter gene into detached cotyledons of *Pinus radiata* was developed and co-cultivation parameters were improved to maximise transient expression of this reporter gene. Techniques to wound cotyledons prior to inoculation with *Agrobacterium* were evaluated. Wounding via any method increased transient *uidA* expression. Although *uidA* expression was highest in cotyledons which had been bombarded with gold or cut diagonally relative to vortexed cotyledons, these alternative procedures were labour intensive and had inherent disadvantages. Hence, vortexing became the method of choice as a wounding procedure. The transformation efficiency was further improved if detached cotyledons were pre-cultured on medium containing cytokinin for seven days prior to inoculation with *A. tumefaciens*. Vacuum-infiltration of detached cotyledons with *A. tumefaciens* also considerably increased transient expression of the reporter gene. Evidence for stable integration of the foreign gene in newly regenerated shoots is presented.

## POSTER 11

### IN-VITRO PRODUCTION OF THE AGROBACTERIUM TUMEFACIENS T-COMPLEX FOR BIOLISTIC TRANSFORMATION OF PLANT CELLS

J. Charity, R. Narayan, J. Korlach, R. Moyle and C. Walter

Genetic Engineering for Future Forests, Forest Research, New Zealand

julia.charity@forestresearch.co.nz  
<http://www.forestresearch.cri.nz>

*Agrobacterium* gene transfer involves production of a T-complex within the bacterium and subsequent transfer into the plant cell. The complex consists of a single-stranded DNA molecule (T-DNA), coated with VirE<sub>2</sub> protein molecule bound to the 5'-end of the T-DNA. It is protected against nuclease attack in plant cells and nuclear localisation signals within the VirD<sub>2</sub> and VirE<sub>2</sub> proteins guide the T-complex into the plant cell nuclei. The T-DNA eventually integrates into the chromosome by illegitimate recombination. It has also been suggested that the efficiency of the integration process is dependent on the VirD<sub>2</sub> protein, which is hypothesised to mediate the precise joining of T-DNA to plant DNA at the terminal nucleotide of the T-strand. We have cloned *Agrobacterium tumefaciens* Ti-plasmid derived *virD<sub>2</sub>* and *virE<sub>2</sub>* genes into pQE vectors for over-expression in *E. coli*. The expression vectors contain a sequence to provide for an in-frame 6x Histidine affinity tag at the 5'-terminus of the cloned gene, for single-step purification of the recombinant proteins using NiNTA affinity chromatography. Both *virD<sub>2</sub>* and VirE<sub>2</sub> were sequestered in insoluble fractions in *E. coli* and were purified under denaturing conditions. To verify functions of the IN-VITRO produced *virD<sub>2</sub>* and *virE<sub>2</sub>* proteins, single-stranded DNA binding assays were performed. The purified and renatured *virD<sub>2</sub>* protein, when incubated with 3'-end DIG labelled T-DNA border sequences, catalysed sequence-specific endonuclease. The protein attached covalently to the bottom, but not to the top strand of the border sequence. Furthermore, gel electrophoresis retardation assays were performed for *virD<sub>2</sub>* and *virE<sub>2</sub>* proteins, after complexing with ssDNA. Both caused a retardation of the ssDNA in 1% agarose gels.

A protocol was developed to bind a ssDNA-protein complex to gold particles for biolistic transformation of *Pinus radiata* embryogenic tissues. Initial results will be presented.

## POSTER 12

### DEVELOPMENT OF IN-VITRO TRACHEARY ELEMENTS STUDIED BY CONFOCAL MICROSCOPY

J. Charity, L.A. Donaldson, L. Grace, and N. Cranshaw

Genetic Engineering for Future Forests, Forest Research, New Zealand

julia.charity@forestresearch.co.nz  
<http://www.forestresearch.cri.nz>

Confocal laser scanning microscopy was used to study the development of tracheary elements in xylogenic tissue cultures of *Pinus radiata* D. Don. Tracheary elements developed after 2 weeks of culture on solid growth medium from a mixture of meristematic cells, characterised by prominent condensed nuclei, plastids (amyloplasts) and large vacuoles, and xylem mother cells with dense cytoplasm containing an extensive endoplasmic reticulum. Several stages of development could be recognised including elongation, and secondary wall formation. Two types of tracheary elements were observed, one with helically thickened secondary walls and bordered pits, and a second larger cell type with bordered pits but no helical thickenings. Comparison with primary xylem from young shoots indicated that both cell types were intermediate between primary metaxylem and secondary tracheids. Tracheary elements showed autofluorescent secondary walls which also stained positively for lignin with toluidine blue. Xylogenic cultures are being used by Forest Research as a tool for rapid evaluation of genetically transformed cell lines.

## POSTER 13

### TOWARDS THE GENETIC MANIPULATION OF COMMERCIALY IMPORTANT PASSIFLORA SPECIES

G.O. Cancino, M.I.S. Gill, M.R. Davey, K.C. Lowe, and J.B. Power

Plant Science Division, University of Nottingham, UK

plxgoc@pln1.life.nottingham.ac.uk  
gcancino@javercol.javeriana.edu.co

Efficient shoot regeneration is essential for *Agrobacterium*-mediated gene transfer. Plant regeneration without a callus phase has been established in *P. mollissima* (Cancino et al., 1998) and has been optimised for 2 commercially-important *Passiflora* species, *P. edulis* fv. *flavicarpa* and *P. giberti*. Leaf and root segments from 4-5 week-old axenic nodal segment-derived plants, were used for regeneration studies. Agar (0.8% w/v) solidified MS medium supplemented with 6-benzylamino purine and kinetin at various concentrations (0.5-5.0 mg l<sup>-1</sup>) and combinations was used to induce shoot regeneration from explants. This direct shoot regeneration protocol provides a baseline for the introduction of agronomically important traits, such as those for fruit quality, insect and disease resistance, into *passiflora* by genetic transformation. With this objective in mind, the supervirulent *Agrobacterium tumefaciens* strain 1065, carrying both the beta-glucuronidase (*gus A*) and neomycin phosphotransferase (*nptII*) genes, was used to infect leaf and root segments of *P. edulis*, *P. mollissima* and *P. giberti*. Several factors, including the plant genotype, explant type, bacterial dilution, inoculation and co-cultivation times, were evaluated in relation to transient *gus* gene expression. The effect of sonication in conjunction with *Agrobacterium*-mediated transformation was also assessed and was found to have a positive effect on transient GUS activity in *P. edulis* and *P. mollissima*.

Cancino GO, Davey MR, Lowe KC, Power JB (1998) *J Expl Bot*, 49 (Suppl), p87.



## POSTER 14

### REGENERATION OF HERBICIDE TOLERANT BLACK LOCUST TRANSGENIC PLANTS BY THE SAAT METHOD

I. Arrillaga, and C. Zaragoza

Dpt. Biología Vegetal, Facultad De Farmacia, Universidad De Valencia, Spain

isabel.arrillaga@uv.es

The SAAT method (sonication-assisted *Agrobacterium*-mediated transformation) (Trick and Finer 1997, Transg. Res. 6,329) and the leaf-regeneration protocol described by Arrillaga and Merkle (1993, HortSci 22,942) were applied to obtain herbicide resistant transgenic black locust (*Robinia pseudoacacia*) plants. Experiments were performed in cotyledon explants using different *Agrobacterium* strains: LBA4404 and EHA105 both containing the 35SGUSINTvector and AGL1 strain carrying the bar and GUS genes. In all vectors, the GUS gene is not expressed in *A. tumefaciens* because the insertion of a plant intron in the protein-coding region. Factors influencing transformation were determined by assaying cotyledons for transient GUS expression three days after infection. Factors tested included, bacterial strains, sonication vs. infection, cotyledon age, explant preconditioning and period of time before applying herbicide selection. From the three different strains used, AGL1 showed a higher capacity to infect black locust cotyledons. When this strain was used together with SAAT, 90-100% of the explants showed such a GUS activity that individual foci could not be distinguished. After setting the bacterial strain and infection method, the remained factors were assayed. From these experiments, the selected protocol to black locust transformation included a 4 days preconditioning on regeneration medium, sonication with AGL1 suspension, coculture for three days and transfer to selection medium with phosphinothricin (PPT) and timentine. Two percent of the initial explants produced at least one transgenic shoot. Genetic transformation was confirmed by PCR and chlorophenol red assays (Kramer *et al.* 1993, Planta 190,454). Further analysis will include Southern hybridization and PAT activity.

## POSTER 15

### THE AC-TRANSPOSON FROM MAIZE IS ALSO ACTIVE IN TREES

M. Fladung

BFH, Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, Germany

mfladung@rrz.uni-hamburg.de  
<http://dainet.de/bfh>

The application of modern molecular technologies previously developed on crop plants to forest tree species offers new opportunities for studies on gene regulation and gene expression. In trees traits are of interest which are associated with tree-specific characteristics, such as fruit quality and flower sterility in fruit trees, and wood properties in forest trees. The application of transposable elements (TE's) is not only restricted to those species that contain well characterized transposons, but has also been extended to species in which none or weakly characterized endogenous transposons are known. The present work describes the transfer of constructs carrying the maize transposable element Ac into the genome of aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x *P. tremuloides*). Two reporter gene systems were employed for transformation. In one system the rolC gene was under control of two different promoters, while in the second the iaal gene expression was controlled by one promoter. It has been shown by PCR, sequencing and Southern analysis that Ac excises from its original position and re-integrates somewhere in the aspen genome. This system will be further developed to establish a 'gene-tagging' protocol in a tree species. Currently, this system is adopted to haploid aspen lines.

## POSTER 16

### GENETIC TRANSFORMATION OF EUCALYPTUS GRANDIS X E. UROPHYLLA

L.M. Sartoretto, M.V. Esmeraldo, L.P. Barrueto Cid, and A.C.M. Brasileiro

Laboratório de Transferência de Genes, Embrapa Recursos Genéticos e Biotecnologia, Brazil

anacmb@cenargen.embrapa.br  
<http://www.cenargen.embrapa.br>

Brazil has the largest area of commercial eucalypt (*Eucalyptus* spp.) plantations in the world, that has been estimated at 4 million hectares. Eucalypts are mainly used in Brazil for pulp and paper industries and for energy as charcoal for the iron and steel industry. The development of genetic manipulation techniques, as genetic transformation, may give to the breeders an additional tool to obtain new eucalypts genotypes. Thus, the objective of this project is to develop strategies to efficiently regenerate and transform the tropical hybrid *Eucalyptus grandis* x *E. urophylla* that is currently being used in important reforestation programs in Brazil. Strategies to obtain an efficient regeneration from different young tissues as cotyledons, hypocotyls and leaves were developed. Treatments with thidiazuron stimulated growth of callus and formation of shoot. Regeneration was further improved when explants were exposed to different light conditions and vitamin compositions. In parallel, we have evaluated the *Agrobacterium*-mediated and bombardment genetic transformation systems. For that, we assessed the susceptibility of *in vitro* *E. grandis* x *E. urophylla* plants to different *A. tumefaciens* and *A. rhizogenes* strains. A disarmed version of the most virulent strain has been used in co-cultivation assays to determine the specific parameters to establish a transformation protocol for *E. grandis* x *E. urophylla*. We are now proceeding this work to improve the regeneration combined with an efficient co-cultivation system to establish a properly protocol for eucalypt genetic transformation.

## POSTER 17

### **PARTIALLY TRISOMIC PLANTS PRODUCED VIA SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE AND EFFECT OF ARTIFICIAL TETRAPLOIDIZATION ON THE REGENERATION CAPACITIES**

J.L. Fourré, P. Berger, L. Niquet, O. Charlier, O. Lebbe, C. Courcelle, P. Richir, F. Hakizimana, N. Malgras and P. André

Unité des eaux et forêts, Université catholique de Louvain, Belgium

fourre@efor.ucl.ac.be  
<http://www.ucl.ac.be/>

We have previously shown that chromosome counting of 7 substrains of 5-year-old embryogenic masses revealed important genomic mutations such as monoploidy ( $2n-1=23$ ), trisomy ( $2n+1=25$ ), double trisomy ( $2n+2=26$ ) and tetraploidy ( $4n=48$ ) (Fourré et al. 1997). Some cases of genomic mutation like tetraploidy seemed to be related to an incapacity to mature. To test if tetraploidy per se can explain the loss of regeneration capacities, artificial tetraploidization of several embryogenic masses (6 clones) was performed using Oryzalin as an antimitotic agent. Most of the tetraploid clones were able to produce mature embryos, but the quality of the germinants was too poor to enable plantlet acclimatization. Chromosome counting of embryogenic masses of 10 other clones with different maturation capacities, 4.5 to 17.4 months after initiation, revealed that : - trisomic and monosomic cells are frequently observed even after a few months in culture - no general and constant increase in these genomic mutations frequency was noted between 4.5 to 17.4 months in culture - these genomic mutations do not seem to be more frequent for embryogenic masses that are not able to regenerate mature embryos. Reduction of the maturation rate of some embryogenic masses could be the consequence of either genetic deviations, or a progressive change of the embryo organisation, or a physiological evolution resulting in a diminished capacity in the synthesis of endogenous components required for embryo maturation. Chromosome counting of 240 acclimated plants was performed in 1995 and 1996 and revealed 3 plants (A8, B274 and C1) with genomic anomalies i.e. completely trisomic (C1) or with trisomic buds and diploid roots (A8 and B274) (Fourré et al. 1997). The B274 acclimated plant was the only genomic mutant with a dwarf phenotype. Additional countings of the same plants and other ones were performed later in 1997 and 1998. Globally 292 acclimated plants were analysed by chromosome counting: 223 in root tip cells only, 42 in bud cells only and 27 in both types of cells. The new countings revealed a fourth phenotypically normal plant with genomic anomalies in buds (C5). Buds of A8, B274 and C1 analysed in 1997 and 1998 were no more totally trisomic but mixoploid with a proportion of trisomic and diploid cells. Diploid and trisomic cells were observed in different buds and in a same bud. The four plants with genomic anomalies appeared singly and quite early during the subcultures, they were regenerated from three different clones, respectively 12.9 (C1 and C5), 13.2 (A8) and 23.3 (B274) months after initiation.

This research was supported by the "Région wallonne" Belgium (convention GRAGEF) and the European union (programme FAIR-CT95-0873)

## **POSTER 18**

### **FIELD BEHAVIOUR OF 5 YEARS-OLD NORWAY SPRUCE SOMATIC EMBRYOS**

L. Harvengt, A. Bouvet, F. Gastine, F. Canlet, I. Reymond, and M. Pâques

AFOCEL, Laboratoire des Ressources du Futur- Domaine de l'Étançon, France (LH, FC, IR and MP), Laboratoire de Biométrie- Domaine de l'Étançon, France (AB), Station régionale NE. France (FG)

biotech@afocel.fr

Five clones of somatic embryos were field established at three sites. The shoot height and diameter were measured each year. The extent of bud flushing was scored two times each year. Data showed that, comparatively to seedlings and cuttings used as control, somatic embryos : grew normally and at some site significantly more homogeneously than seedlings. Homogeneity of behaviour is particularly spectacular at the level of bud flushing synchrony with emblings flushing all at the same time while seedlings of the same seedlot showed a very heterogeneous response to environmental conditions. Growth performances are as good as expected relatively to the origin of « mother plants » i.e. equivalent or superior to seedlings for clone produced from respectively unselected seedlings (immature embryos and plantlets) or selected plants (old trees).

## POSTER 19

### SCOTS PINE AS A TARGET FOR MOLECULAR BREEDING

T.S. Aronen, T.O. Nikkanen, and H. M. Häggman

Punkaharju Research Station, Finnish Forest Research Institute

tuija.aronen@metla.fi

<http://www.metla.fi/pu/indexe.htm>

In 1990's, Scots pine (*Pinus sylvestris*) has been a target for biotechnological studies in order to find new means for facilitating classical breeding, i.e. an effective method for vegetative propagation and genetic transformation. In our lab, two tissue culture systems have been developed for Scots pine; a method based on organogenesis using cotyledons excised from germinated embryos as explants, and somatic embryogenesis starting from immature female gametophytes. More research is, however, needed to get a technique applicable to practical forestry or breeding. Success of both methods is genotype-dependent, and plantlets regenerated through organogenesis tend to show growth habit problems and early flowering. Transformation studies using both regenerating cotyledons and embryogenic cultures as targets for particle bombardment have been carried out. Embryogenic cell lines stably transformed with reporter genes have been obtained, but regeneration of transgenic Scots pine plants have not yet succeeded. As an alternative for tissue culture material, pollen has been subjected to particle bombardment, and a specific technique developed for dehydrating and storing bombarded pollen suspensions to be used in controlled pollinations. To date, 267 seedlings have been obtained from these crossings and examined for reporter gene expression without reliable evidence of transgenic progenies. Around 8900 seeds with bombarded pollen as one parent are, however, still to be sown and tested in this year. Besides the recent progress of the transformation studies, the potential target traits in Scots pine and problems involved in the integration of genetic engineering into classical breeding will be discussed.

## POSTER 20

### ADVENTITIOUS SHOOT REGENERATION FROM SOMATIC TISSUES OF WILD CHERRY (*PRUNUS AVIUM* L.)

A. De Rogatis

Istituto Sperimentale per la Selvicoltura (MIPA), Italy

fulvio@krenet.it

In the framework of genetic improvement and breeding of some noble hardwood species, the adventitious shoot regeneration from somatic tissues (cotyledon and leaf) of *Prunus avium* L. was studied. The research on cotyledon tissue tested nine clones (VTN04, VTN03, VTS01, VTS02, ACW03, ACW10, BF04, TNW04, FL02), the basal media MS, MB, DCR, DKW, the growth regulators IAA, NAA, 2,4-D, ZEA, KIN, BAP, different maturation phases of cotyledons. Only immature cotyledon produced shoot regeneration. VTN04, VTS01, VTN03 gave the 100% regeneration frequency on MS with BAP and IAA or 2,4-D (0,5-5 mg/l), ACW10, ACW03 on DCR with IAA and BAP (2-8 mg/l), BF04 on MB with IAA and BAP (2-4 mg/l), ACW10 the 80% regeneration frequency on DCR with IAA and BAP (1-5 mg/l). Significant differences were among the genotypes and among the growth regulators, and significant interaction were between these two factors. The research on leaf tissue tested seven micropropagated clones (PC, CA1, VTS02, ACW03, VM02, FL02, FL07), the basal media MS, DCR, WPM, different explant dimensions, the growth regulators IAA, NAA, ZEA, 2,4-D, BAP and TDZ, different containers to be use before the induction. AC1 and VM02, grew into coulter containers, produced 75% regeneration frequency on DCR with TDZ (0,2-2,5 mg/l) with leaf dimensions 0,5-1 mm, CA1 gave the best mean number of shoots for explant, the best medium was DCR, but the best for PC was WPM.

## POSTER 21

### DEVELOPMENT OF TRANSGENIC POPLARS CARRYING A TADPOLE FERRITIN cDNA

Y.I. Choi, E.W. Noh, H.K. Moon, S.H. Son, E.R. Noh, and M.S. Yang

Biotechnology Division, Forestry Research Institute(YIC, EWN, HKM, ERN, SHS),  
Department of Molecular Biology, Chunbuk National University (MSY)

cyim@Hitel.net

Plant-based system for phytoremediation has been under serious consideration to remove soil pollutants. We have attempted to enhance their pollutant absorbing capacity by transfer of a metal binding protein gene to a hybrid poplar clone (*Populus alba* x *P. glandulosa*). A ferritin cDNA was synthesized from tadpole mRNAs and cloned into a plant expression vector pBI121. The transgenic poplars were regenerated after *Agrobacterium*-mediated transformation. The transformants were confirmed by PCR, Adaptor-aided PCR, Southern hybridization and RT-PCR, and then transferred to the greenhouse to test their tolerance to cadmium and capacity of holding cadmium in the tissue. The plants were grown hydroponically in the presence of varying levels of cadmium. The difference between the transgenic plants and control plants was detected only at the highest level (0.5 mM) of cadmium. The same results were obtained in the test tube experiments using both callus inducing media and shoot inducing media in the presence of cadmium. The transgenic plants grew consistently better at the higher concentration. The ICP assay of the plants grown in the presence of 0.01mM cadmium revealed that some transgenic clones contained about three times more cadmium than did control plants. Other interesting observations included that 1) both transgenic and control plants grew better at 0.1 mM than any other levels of cadmium including control, and 2) transgenic and control plants differ in their response to cadmium illustrated by changes in cellular polyamine contents.



## POSTER 22

### **BASTA RESISTANCE AS A SELECTABLE AND SCREENING MARKER FOR TRANSGENIC NORWAY SPRUCE**

V. Brukhin, M. Elfstrand, D. Clapham, and S.von Arnold

Department of Forest Genetics, SLU, Uppsala, Sweden

Vladimir.Brukhin@sgen.slu.se

Embryogenic cell-lines of Norway spruce are sensitive to the herbicide Basta at low concentration, 1 mg/L, corresponding to 0.2 mg phosphinothricin/L. We have developed a particle bombardment method for the transformation of embryogenic Norway spruce cultures in which the bar gene coding for resistance to Basta fused to the maize ubiquitin promoter is the selectable marker (Clapham et al. submitted). Basta resistance has now been used as a screening marker for transgenic plantlets. Ten fully elongated needles were removed from each nine-month old plantlet derived from transgenic cultures or untransformed controls and placed on filter-papers soaked in a solution containing Basta at 500 mg/L, Tween 0.1%. After 2 weeks at 20°C, over 90% of the needles from the transformants remained green while 90% of the control needles had yellowed, with little change after one month. No transgenic plantlet of the 43 tested showed as many yellowed needles as in the controls. Needles are much less sensitive to Basta than embryogenic cells; untransformed needles did not yellow at concentrations below 50 mg/L. The polymerase chain reaction (PCR) using primers to the bar gene confirmed the presence of the transgene in the genomes of the putatively transformed plants.

## POSTER 23

### IN VITRO ADVENTITIOUS SHOOTS REGENERATION FROM LEAVES OF *SORBUS AUCUPARIA*

S.V.Dolgov, E.V.Alimova, A.P.Firsov.

Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS. 142292 Pushchino Moscow region, Russia

Successful *in vitro* micropropagation of four *Sorbus aucuparia* varieties has been achieved. Explants were taken from mature trees of five years old. The best results were obtained on QL media in addition 1 mg/l QL vitamins, 1 - 1,5 mg/l BA, 0,2 mg/l IBA, 0,5 mg/l GA<sub>3</sub>, 20 g/l sucrose and 7 g/l Bactoagar. The multiplication rates were 5 - 6 for 'TITAN' variety, and 7 - 10 for 'LIQIORNAYA' variety during 45 days. Explants rooted on half-strength QL media containing 0,5 mg/l MS vitamins and 0,5 mg/l IBA. Rooting capacity of 'TITAN', 'LIQIORNAYA', 'GRANÁT NAYA' varieties was about 90 - 100 %. Efficient regeneration system from leaves disks have been developed. The best results were achieved for 'TITAN', 'LIQIORNAYA' varieties on MS media supplemented with thidiazuron (TDZ) as cytokinin and other hormones, such as NAA, GA<sub>3</sub>, vitamins QL and high concentration of sucrose. The regeneration frequency from leaf explants of 'TITAN' variety was 23 - 25%, and 'LIQIORNAYA' variety - 35 - 38%, with 1 - 2 plantlets formation on one leaf disk. Obtained results can be used for further biotechnology application in *Sorbus aucuparia* breeding.

## POSTER 24

### THE PLANT REGENERATION FROM EXCISED COTYLEDONS OF PINUS RADIATA

K.A. Shestibratov, S.V. Dolgov

Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS. 142292 Pushchino Moscow region, Russia

Plant genetic-transformation techniques and gene isolation and characterization are no longer serious problems; forest-tree species should be a major target for commercial genetic engineering and molecular breeding. For conifers, a most important group of forest-tree species, reliable and efficient methods of *in vitro* regeneration and clonal propagation are still lacking, and this is a serious obstacle to their genetic engineering. Two efficient regeneration protocols from somatic tissue via organogenes have been developed for *Pinus radiata*. Excised cotyledons from germinated seeds were used as an explant. At the first protocol initiation of adventitious shoots on explants was carried out on a SH medium containing 3% sucrose and supplemented with 5 mg/l BAP for 3 weeks. For subsequent transfers, which were carried out every 3 or 4 weeks, the tissues were placed on the same nutrient medium but containing only 2 % sucrose. The frequency of adventitious shoots formation was about 85-90 %. At the second one numerous meristematic nodules were induced when excised cotyledons were placed on a \_ LP medium containing 5 mg/l BAP. Half-strength LP medium proved best for the first 6 weeks in culture, whereas LP medium proved best after six months in culture. The frequency of nodules formation was about 85-90 %. These efficient regeneration systems may be useful for genetic transformation of *Pinus radiata*.

## POSTER 25

### CHARACTERISATION OF A HOMEBOX GENE EXPRESSED DURING SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE

M. Ingouff and S. von Arnold.

Forest Genetics, Swedish University of Agricultural Sciences

mathieu.ingouff@sgen.slu.se

In our attempts to understand the regulation of early embryo development in Norway spruce, we are using embryogenic cultures. The embryogenic culture contain embryos which have reached various developmental stages. We are focusing on homeobox genes expressed during Norway spruce embryogenesis. These master control genes have been found to play important roles in developmental processes (control of cell specification and pattern formation) in both animal and plant kingdoms. Plant homeobox genes have been isolated and grouped into classes based on homeodomain sequence similarity and additional conserved protein motifs outside the homeodomain. The homeobox gene ATML1 (*Arabidopsis thaliana* meristem L1 Layer) was shown to be specifically expressed in the protoderm in embryos and in the L1 layer of the seedling shoot apical meristems of *Arabidopsis*. Based on sequence similarities, ATML1 and the homeodomain proteins, GLABRA2 (GL2) in *Arabidopsis* and O39 in orchids were classified as the HD-GL2 (Homeodomain-GL2) family. An additional member to this class was also isolated in sunflowers, HAHR1 (*Helianthus annuus* homeobox from roots). It is suggested that ATML1 and GL2 are involved in epidermal cell differentiation events in *Arabidopsis*. I isolated and characterised a cDNA designated Pa-HB1 (for *Picea abies*-Homeobox1). The Pa-HB1 gymnosperm protein shares high homology with HD-GL2 angiosperm proteins, but much less homology with representatives of the other classes of plant homeodomain proteins. It is a new member of the HD-GL2 family. Pa-HB1 expression is detected in reproductive, vegetative and somatic embryogenic tissues. Pa-HB1 spatial expression pattern during spruce embryogenesis and seedling development will also be discussed.

## POSTER 26

### PLASTICITY OF LIGNIN SYNTHESIS AND ITS EFFECT ON PULPING IN CAD-DEFICIENT PINE

R. Sederoff, J. Mackay, C. Lapierre, B. Pollet, D. Dimmel, E. Althen, C. Parks, J. Scott, and D. O'Malley

Forest Biotechnology Group, North Carolina State University (RS, JS AND DO), Institute Of Paper Science And Technology (JM, DD, EA, CP), Laboratoire De Chimie Biologique Inra, Institut National Agronomique (CL, BP)

volvo@unity.ncsu.edu  
john.mackay@ipst.edu

A mutant allele (*CAD-N1*) of the gene encoding cinnamyl alcohol dehydrogenase (CAD) was used to probe the potential for genetic modification of lignin in CAD-deficient pine. Previously, we showed that lignin isolated from a *CAD-N1* homozygous tree incorporated dihydroconiferyl alcohol (DHCA) as a major component. Here, the incorporation of DHCA is demonstrated by thioacidolysis run on *in situ* lignin of several *CAD-N1* homozygous loblolly pines of different ages and grown in various conditions. Other alterations revealed or confirmed by thioacidolysis include increased incorporation of coniferaldehyde and vanillin, a doubling of free phenolic groups and a distinct distribution of interunit linkages, reflected by a severely reduced thioacidolysis yield and an unusual distribution of lignin-derived dimers. The quantification of thioacidolysis resistant dimers (several newly described structures) from CAD-deficient wood shows that the lignin contains fewer ether linkages and more carbon-carbon linkages. Finally, woods from *CAD-N1* heterozygous trees (partially CAD-deficient), analyzed with the DFRC method showed a 50% decrease in beta-O-4 linked guaiacyl units indicating that partial CAD deficiency may also alter lignin structure in pine. These data clearly show that lignin biosynthesis exhibits considerable metabolic plasticity in pine, suggesting that opportunities to modify lignin may be greater than previously anticipated. Furthermore, the altered linkage types of lignin in CAD-deficient pine could be expected to impede delignification, however lignin removal was increased with mild kraft and soda pulping conditions. The increase in lignin reactivity may be explained by the lower average molecular weight, observed in milled wood lignin isolated from CAD-deficient wood.

## **POSTER 27**

### **MANIPULATING COMBINATIONS OF LIGNIN GENES IN TOBACCO**

A. Barakate, A. O'Connell, W. Schuch and C. Halpin.

Dept. Biological Sciences, University of Dundee (AB, CH) and Zeneca (AO'C, WS)

a.barakate@dundee.ac.uk

A number of reports have shown that lignin composition and content can be altered by reducing expression of lignin biosynthetic genes. The exact nature of the changes depend on the particular gene targeted. Some of the changes in these modified lignins are difficult to explain by reference to the accepted pathway for lignin biosynthesis. Down-regulation of multiple genes may help us to explain these anomalies. We used a single-step strategy to down-regulate different combinations of cinnamyl alcohol dehydrogenase (CAD), cinnamyl-CoA reductase (CCR) and O-methyltransferase (OMT) in tobacco. Plants with low CAD+CCR or OMT+CCR activities have been produced using single chimeric transgenes. Compared to the wild-type, these transgenic plants show differences in lignin content/composition and in development. Further analysis is needed to study the nature of these changes. We now have a library of plants down regulated in all the combinations of CAD, CCR and OMT. This material is currently being used to study the regulation of the lignin biosynthetic pathway and its role in plant development. We hope that analysis of these plants will help to elucidate the exact sequence of reactions and identity of enzymes functioning in lignin monomer biosynthesis. Our work will also determine whether further useful improvements can be made to lignin.

## POSTER 28

### STRATEGIES FOR SIMULTANEOUS DOWNREGULATION OF CAD AND OMT IN TOBACCO

J.Abbott, A. Barakate, W. Schuch, G. Pinçon, M. Legrand, and C. Halpin

Dept. Biological Sciences, University of Dundee, UK (JA, AB, CH), Zeneca (W.S) and IBMP Strasbourg, France (GP, ML)

j.abbott@dundee.ac.uk

A number of recent reports have demonstrated that downregulation of lignin biosynthetic enzymes can lead to changes in the content and composition of lignin, in some case increasing the extractability of the lignin. These changes are not, however, always in accordance with the previously accepted model of the biosynthetic pathway. We are working to downregulate combinations of these enzymes to try to elucidate further information on the pathway structure. Two of our target enzymes are cinnamyl alcohol dehydrogenase (CAD) and caffeic acid o-methyltransferase (COMT), and we have produced plants downregulated in both enzymes. Attempts to downregulate multiple genes in plants frequently run into difficulties as a result of gene silencing, which causes a loss of transgene expression. Some methods for downregulating multiple genes are also highly time consuming i.e. re-transformation of plants downregulated in one enzyme with a second transgene. We are comparing the use of three different systems for downregulation of CAD and COMT simultaneously; crossing plants downregulated in single genes, the use of double partial sense constructs, and the use of a potato virus X vector (collaboration with Van Der Have). In addition, we are also using the partial sense strategy to downregulate CAD, COMT and cinnamoyl-CoA reductase (CCR) simultaneously.

## POSTER 29

### ARABIDOPSIS LACCASES

C. Halpin, A. Barakate, J. Abbott, and A.El Amrani

Dept. Biological Sciences, University of Dundee, UK (CH, AB, JA), INRA Versailles, France (AEA)

c.halpin@dundee.ac.uk

The controversy concerning the identity of enzymes responsible for the polymerization of lignin monomers is still unresolved. Both peroxidases and laccases are candidate enzymes and similar evidence can be cited to support a role for either in lignin biosynthesis. In general such evidence is circumstantial eg. temporal and spatial coincidence of enzyme expression in tissues actively depositing lignin. Clones encoding plant peroxidases have been available for some time and transgenics overexpressing or underexpressing particular isozymes have been produced. Such experiments have not yet produced unequivocal data supporting a role for peroxidases in lignin polymerization. The multiplicity of peroxidase genes present in higher plants complicates such experiments so that no firm conclusions can be drawn. By contrast few laccase clones have yet been isolated and no reports of genetic manipulation of laccase expression exist in the literature. A number of groups are reportedly performing such work in woody plants. Producing transgenics in tree species can be a lengthy and laborious process. We have therefore chosen the more rapid route of investigating and manipulating laccase expression in *Arabidopsis*. By searching all available *Arabidopsis* sequences, we have been able to identify a number of distinct laccase genes and have made transgenics expressing antisense or partial sense sequences for one of these. Some of our transgenics display aberrant phenotypes. We are currently performing a variety of analyses to determine whether or not lignin is altered in these plants.



## POSTER 30

### IS OZONE TOLERANCE CORRELATED WITH THE LEVEL OF CAD ACTIVITY IN POPLAR TREES ?

M. Cabané, F. Froux, J-C. Pireaux, E. Weber, and P. Dizengremel

Laboratoire de Biologie Forestière, Université Henri Poincaré, France

[cabane@scbiol.u-nancy.fr](mailto:cabane@scbiol.u-nancy.fr)

Previous studies showed that several enzyme activities involved in the lignin biosynthesis pathway were increased under ozone treatment. In this work we examined the possible role of CAD activity (cinnamyl alcohol dehydrogenase, enzyme catalysing the last step of monolignol synthesis) in ozone tolerance. Poplar trees were fumigated for 41 days with ozone (100 ppb) during the light period in phytotronic chambers. Three poplar clones were fumigated : T21 and T52 (two CAD-down-regulated plants) and NT (a wild-type plant). Enzyme activities and RNA levels were measured in old leaves, young leaves and in stems. The effects of ozone were examined on cinnamyl alcohol dehydrogenase (CAD) as well as two other enzymes of importance in the link between primary and secondary metabolism: shikimate dehydrogenase (SHDH) and phenylalanine ammonia lyase (PAL). Under ozone treatment, CAD activity was more stimulated in T21 leaves than in T52 leaves. Nevertheless, damages caused by ozone were similar in both T21 and T52 clones. These results suggested that CAD does not play a major role in ozone resistance. Moreover, in the two CAD-down-regulated plants a lower PAL activity was measured, suggesting a strong coordination in the regulation of the two enzymes.

## POSTER 31

### **GENETIC MODIFICATION OF LIGNIN BIOSYNTHESIS IN POPLAR TO IMPROVE WOOD QUALITY FOR THE PULP AND PAPER INDUSTRY**

M. Baucher, J. H. Christensen, H. Meyermans, C. Chen, J. Van Doorselaere, J.-C. Leplé, G. Pilate, C. Lapierre, B. Pollet, L. Jouanin, M. Petit-Conil, M. Van Montagu and W. Boerjan.

Plant Biotechnology, University of Brussels, Belgium (MB), Plant Genetics, VIB-University of Ghent, Belgium ( JHC, HM, CC, JVD, MVM, WB), INRA-Orléans, France (JCL, GP), INRA-Grignon, France (CL, BP), INRA-Versailles, France (LJ), CTP, Grenoble, France (MPC)

[mbaucher@ulb.ac.be](mailto:mbaucher@ulb.ac.be)

<http://www.plantgenetics.rug.ac.be/>

During chemical pulping, lignin has to be removed from cellulose. This process is toxic, energy consuming, and results in a low biomass utilization. For the pulp industry, it would be beneficial to process trees which have either less lignin, or a modified lignin that is easier to separate from cellulose. Through genetic engineering, we have modified the expression of genes that play crucial roles in lignin biosynthesis in poplar, i.e. those encoding caffeic acid/5-hydroxyferulic acid-O-methyltransferase (COMT), caffeoyl-CoA-O-methyltransferase (CCoAOMT), cinnamoyl-CoA-reductase (CCR), cinnamyl alcohol dehydrogenase (CAD) and putative lignin-specific peroxidases, and analysed the impact on Kraft pulping. Inhibition of the COMT activity in transgenic poplar results in a significant change in the composition of lignin with the simultaneous appearance of a novel lignin unit. Due to the more condensed lignin in these transgenic trees, the lignin is more difficult to extract during chemical pulping, as indicated by the higher kappa number. In contrast, inhibition of CAD activity in poplar results in different lignin characteristics yielding an increased lignin extractability during chemical pulping. The effect of a down-regulation of CCoAOMT is a reduction in the amount of lignin as well as a lower kappa number. The analysis of transgenic poplars with modified CCR and peroxidase levels is underway and field trials for antisense CAD and antisense COMT plants are running. This work was carried out in the framework of the EU Research Programs AGRE-0021-C, FAIR-PL 95424, and AIR2-CT93-1661.

## POSTER 32

### FORMATION AND CHARACTERIZATION OF TRANSFORMED WOODY PLANTS INHIBITED LIGNIN BIOSYNTHESIS

Y.Tsuji,S.Kawai,Y.Katayama,N.Morohoshi, K.Yamada,H.Ebinuma

Graduate School of Bio-application and Systems Engineering,Tokyo University of Agriculture and Technology, Japan (YT,YK,NM), Faculty of Agriculture,Tokyo University of Agriculture and Technology, Japan (SK) and Central Research Laboratory,Nippon Paper Industries, Japan (KY,HE)

tujigiri@cc.tuat.ac.jp

We focused peroxidase that is one of the enzymes catalyzing the polymerization of monolignols. We have analyzed the expression of some peroxidase genes isolated from hybrid aspen and a peroxidase gene (PRXA3A) was determined as a gene encoding the peroxidase isozyme involved in lignin biosynthesis (K.Osakabe et al. 1994). We transformed poplars transformed with a PRXA3A antisense RNA expression vector having an original promoter region of this gene. These transformants were subjected to some analyses, enzyme assays, lignin analyses, carbohydrate analysis and so on. We present the results of these analyses about in vitro transformants. We got the results that peroxidase activities of transformants were lower than that of wild type poplars and that an anionic peroxidase isozyme was drastically suppressed in transformants on IEF patterns. These results show that the expression of PRXA3A gene is suppressed by the antisense RNA method. These transformants were subjected to the acetyl bromide method, the potassium permanganate oxidation and the thioacidolysis. We got the results that lignin contents in transformants were smaller than lignin contents in wild poplars and these transformants have much more arylglycerol-beta-arylether structures than wild poplars. In addition to these lignin analyses, the alditol acetate method was performed as a carbohydrate analysis. Some transformants increased glucose and decreased xylose contents in comparison with the wild. The above results will give us a clue to elucidating the role of peroxidase in lignin biosynthesis and possibilities that useful tree is made by genetic engineering techniques in the future.

## POSTER 33

### EVALUATING THE SOD ACTIVITY AND CHLOROPHYLL FLUORESCENCE IN SPRUCE GROWING AT DIFFERENT ALTITUDES

E. Niewiadomska, E. Kepa, P. Skawinski and Z. Miszalski

Department of Plant Physiology, Polish Academy of Sciences

E.Niewiadomska@zfr.pan.krakow.pl

SOD activity and parameters of chlorophyll fluorescence:  $F_v/F_m$ ,  $F_m$  and  $F_o$  were determined on *Picea abies* growing at different altitudes. The decrease of  $F_v/F_m$  ratio and of  $F_m$  were measured on trees from the highest stands. The decrease of fluorescence parameters was reversible, at least partly, after keeping the branches for some days in the laboratory conditions. The ratio measured in spring, when trees were partially covered with snow, revealed greater degree of photoinactivation in branches collected from above the snow in comparison to those from below the snow. In samples collected from above snow also slower recovery from stress was observed. Partial photoinhibition is discussed as a photoprotective strategy of adaptation to excess light condition. Two main SOD isoforms were determined in needles of *Picea abies*, and classified as CuZnSOD. The activity of both SOD isoforms was increasing with the altitude, thus indicating the highest level of oxidative stress at the timberline zone.

## POSTER 34

### EXPLORING THE CONTROL OF MERISTEM ACTIVITY IN EUCALYPTUS

H.M. Jones, K. Meyer, and M.M. Campbell

Plant Sciences, University of Oxford, UK (HMJ, MMC) and Shell Forestry, UK (KM)

[helen.jones@plants.ox.ac.uk](mailto:helen.jones@plants.ox.ac.uk)  
<http://users.ox.ac.uk/~dops0022>

The shoot apical meristem (SAM) plays an important role in determining the form and rate of growth of all plants. As a result there has been a great deal of interest in determining the mechanisms that control meristem activity in plants. Recently, genetic analysis has provided an insight into the control of meristem activity. The identification of mutant ARABIDOPSIS THALIANA (arabidopsis) plants defective in some aspect of SAM function has enabled the cloning and characterisation of a number of genes involved in the development of the SAM. One important gene identified was the *SHOOT MERISTEMLESS* (*STM*) gene, which is required for both SAM initiation and maintenance. *STM* belongs to the *KNOTTED*-like homeobox (*KNOX*) class of genes. The *KNOTTED-1* (*KN1*) gene was isolated by transposon tagging a dominant leaf mutant of maize and was amongst the first of the plant homeobox genes to be cloned. The homeobox sequence of *KN1* was used to isolate further genes from arabidopsis. *KNAT1* and *KNAT2* (*KNOTTED*-like From *Arabidopsis thaliana*) are just two of such genes. *KN1*, *STM1*, *KNAT1* and *KNAT2* belong to the class-1 homeobox genes due to their sequence similarities and their expression predominately in the meristematic region. Until recently it was not known whether the molecular mechanisms controlling meristem initiation and maintenance were conserved in woody species. This project will attempt to identify putative orthologues of genes controlling meristem function in the economically important woody perennial *Eucalyptus*.

## POSTER 35

### EXPANSIN GENE EXPRESSIONS DURING ADVENTITIOUS ROOT FORMATION IN LOBLOLLY PINE (PINUS TAEDA L.)

S.M. McInnis, F. Xu, M.S. Greenwood, and K.W. Hutchison

Plant Sciences, University of Oxford (SMM) and Forestry, University of Maine (FX, MSG) and Biochemistry, University of Maine (KWH)

stephanie.mcinnis@plants.ox.ac.uk  
<http://www.ume.maine.edu/~bmmb/KWH.html>

Expansins are a family of extracellular proteins, initially characterised in angiosperms, which function to increase primary wall extensibility during plant cell elongation. Maturation-related loss of adventitious rooting capacity of cuttings is common in conifers, and occurs particularly early in loblolly pine seedlings. When exposed to IBA or NAA, cuttings from hypocotyls root well within 20-30 days while epicotyl cuttings from the same seedlings root infrequently after 60 days. This abrupt decline in adventitious rooting has hindered the application of clonal techniques in loblolly. While searching for auxin-induced genes specific to adventitious rooting, we found that auxins induce expansins, which were highly conserved in sequence. To investigate the temporal relationship of expansin expression to adventitious root meristem formation, auxin-induced expression of the expansin gene was studied in 25-day-old hypocotyls and 50-day-old hypocotyls and epicotyls. *In situ* hybridization revealed precise localization of expansin mRNA to parenchyma cells from which adventitious roots arise in 25-day-old auxin-treated hypocotyls, while no localized expression was observed in water-treated controls. Peak expression occurred at 48 hours, NPA blocks rooting and inhibits expansin expression if applied prior to this time. RT-PCR revealed that members of the loblolly expansin gene-family were differentially expressed during the early stages of root initiation. Though expansins are induced by auxins in both hypocotyls and epicotyls, Northern blots and RT-PCR reveal lower levels of expression in epicotyls.

## POSTER 36

### COMPARISON OF MYB GENE FUNCTION BETWEEN A HERBACEOUS ANNUAL, *ARABIDOPSIS THALIANA* AND A WOODY PERENNIAL, *EUCALYPTUS*

L. Juda, L.J. Newman and M.M. Campbell

Plant Sciences, Oxford University

lusanda.juda@plants.ox.ac.uk  
<http://users.ox.ac.uk/~dops0022/>

MYB proteins are transcription factors recognised by their conserved DNA binding domain. In plants, MYB proteins represent a relatively large family of transcription factors. For example, in *Arabidopsis thaliana*, over 100 different *MYB* genes have been identified. It is hypothesised that MYB factors are used to direct a diverse array of transcriptional activation events in diverse tissues. The mechanisms underlying the deployment of specific MYB proteins in specific tissues are not well characterised. As a first step in examining the evolution of MYB gene regulation, studies have been initiated on genes encoding MYB proteins from two evolutionarily diverse plant species; a herbaceous annual, *Arabidopsis thaliana* and a woody perennial, *Eucalyptus*. We report on progress towards understanding the regulation of *MYB* genes from *Arabidopsis* and *Eucalyptus*. The promoters of the *MYB* genes from these two plant species are being cloned upstream of the *uidA* gene to drive its expression. The constructs are being expressed in transgenic *Arabidopsis* plants and the plants analysed for the expression of beta-glucuronidase. These experiments will provide insights into the spatial and temporal expression of MYB genes. Ultimately these promoter studies should provide comparative information and highlight differences of the mechanism of MYB gene regulation between two evolutionarily distinct plants.

## POSTER 37

### PROTEINS IN OVULAR SECRETIONS OF DOUGLAS FIR

Brett Poulis & Patrick von Aderkas

University of Victoria, Canada

poulis@uvic.ca

In Douglas fir (*Pseudotsuga menziesii*) a postpollination/prefertilization ovular secretion fills the micropylar canal. This secretion can be collected for analysis from dissected female cones. Protein analysis of this drop by sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) shows the presence of approximately sixteen proteins with molecular weights ranging from 130 kDa to 12 kDa. The proteins were separated and collected in individual fractions using Reverse Phase - High Performance Liquid Chromatography (RP-HPLC) which separates proteins based on their relative hydrophobicities. The majority of the proteins eluted off the C-8 (2.1mm) column when the percentage of acetonitrile was in the range of 35-50%. This represents the first protein analysis in ovular secretions of gymnosperms.



## POSTER 38

### PROTEINS IN OVULAR SECRETIONS OF HYBRID LARCH

Stephen J. O'Leary & Patrick von Aderkas

University of Victoria, Canada

[sjoleary@uvic.ca](mailto:sjoleary@uvic.ca)

In hybrid larch (*Larix kaempferi* X *L. decidua*) a secretion fills the micropylar canal of the ovule during a brief period coinciding with sexual maturation of the egg. The secretion contains a variety of compounds including proteins. These proteins range in mass from 12 - 97 kDa, as determined by SDS-PAGE. Run in one dimension, there are approximately 12 bands clearly resolved. However, 2-D gel electrophoresis indicates that some proteins are present in a number of isoforms. Reverse Phase HPLC also indicates that the protein complement of the drop is both substantial and complex. This work represents the first report of proteins in the ovular secretions of any higher plant. The future aim of this research is to study the proteins present in the ovular secretions of gymnosperms for possible roles in pollen selection, pollen tube development, and other events leading to fertilization.

## POSTER 39

### CONSTITUTIVE ECTOPIC EXPRESSION OF THE ARABIDOPSIS THALIANA AUX1 GENE IN PRUNUS PADUS REDUCES ROOTING COMPETENCE

N. J. Grant and N. Hammatt

Horticulture Research International, UK

<http://www.hri.ac.uk/site2/woodland.htm>  
neil.grant@hri.ac.uk

Poor rooting competence hinders development of clonal forestry because supporting tree improvement programmes rely on vegetative propagation to preserve superior genotypes. They also typically require the propagation of mature woody plants, which are more difficult to propagate vegetatively than juvenile counterparts. Rooting may depend on cellular competence to respond to root-inducing stimuli, particularly auxins. We tested the hypothesis that transformation of *Prunus padus*, a relatively easy-to-regenerate model woody species, but recalcitrant in adventitious rooting, with the *Arabidopsis* AUX1 gene (a putative cellular auxin influx carrier), may enhance the delivery of the auxin root-inducing signal and improve rooting competence. AUX1 was under constitutive 35S CAMV (cauliflower mosaic virus) promoter control. Polymerase chain reaction analysis suggests that six independently transformed shoot lines have the complete AUX1 gene; these await further molecular analyses. Transgenic shoots have reduced rooting competence, which could be improved by increasing auxin concentration, suggesting the effect was not due to inhibition of root outgrowth. This effect appears to be independent of proposed auxin affinities for the influx carrier. Results may be explained if ectopic AUX1 expression promotes auxin uptake by cells irrespective of their competence to form roots; thus, more endogenous auxin may be needed to reach competent cells. Alternatively, the transgene may suppress native AUX1 gene expression. If co-ordinated auxin movement is disrupted, use of AUX1 to improve rooting may require more targeted expression of the transgene using tissue-focused promoters.

Acknowledgement: This work was funded by the Biotechnology and Biological Sciences Research Council.

## POSTER 40

### AGROBACTERIUM-MEDIATED TRANSFORMATION AND REGENERATION OF HYBRID POPLAR EXPRESSING A BACTERIAL SALICYLATE HYDROXYLASE GENE

P.M. Pijut and J. M. Davis,

North Central Research Station, USDA Forest Service (PMP) and School of Forest Resources and Conservation, University of Florida (JMD)

pijut001@maroon.tc.umn.edu

A hybrid poplar clone, INRA 717-1B4 (*Populus tremula* X *Populus alba*), was transformed with the salicylate hydroxylase gene (*nahG*) in order to assess the importance of salicylic acid in disease resistance of *Populus*. The *nahG* product converts salicylic acid to catechol, a compound that is not a physiological inducer of disease resistance responses. Transformants were obtained using *Agrobacterium tumefaciens* strain C58pMP90 (a disarmed derivative of the nopaline C58 strain) and plasmid pCIB200 containing *nahG* regulated by a CaMV 35S promoter and a selectable gene-encoding neomycin phosphotransferase II (*nptII*). Transformed cells from co-cultured leaf explants were selected and shoots were regenerated in the presence of 100 milligrams per liter kanamycin. Shoots were rooted in the presence of 25 milligrams per liter kanamycin, transferred to a 1 perlite : 2 peat potting mix and slowly acclimatized to the greenhouse environment. DNA (PCR) and RNA (Northern blot) analysis confirmed the presence and transcription of the *nahG* transgene in 24 out of 26 independent regenerants. Using these transformed *nahG* plants we will compare the disease resistance of normal and transgenic plants. If salicylic acid is required for conditioning poplar defense responses to certain pathogens, then resistance to those particular diseases should be impaired in the transgenic plants. Others have used this approach to show that salicylic acid is required for resistance to certain diseases in tobacco and *Arabidopsis*.

## POSTER 41

### EFFECTS OF WATER DEFICIT ON GROWTH AND EXPRESSION OF THE BACTERIO-OPSIN GENE IN TRANSGENIC HYBRID POPLAR

C.H. Michler, S.A. Netzer, R. Mohamed, and P.D. Anderson

USDA Forest Service, North Central Research Station (CHM, SAN, PDA) and Department of Forest Science, Oregon State University (RM)

cmichler@fnr.purdue.edu

The bacterio-opsin (bO) gene from *Halobacterium halobium* is known to increase production of phytoalexins and pathogenesis related proteins and produce a phenotype in tobacco and arabidopsis with foliar lesions that mimic those produced with the hypersensitive response. We tested the effectiveness of drought stress for induction of foliar lesions as a portion of a program to test the effectiveness of this transgene in hybrid poplar disease resistance (*P. Trichocarpa* x *P. deltoides* 195-529). Results will be presented from tests of two transgenic lines compared to the non-transformed parent clone; one with low transcription (#26-7) and one with high transcriptional levels (#18-3) of the transgene. For experimentation four 10" cuttings of each transgenic line were rooted in two gallon pots in RediEarth. Trees were kept well watered until they reached an average plastocron index of 24. Trees in the water deficit treatments were allowed to dry to 13% volumetric water content (vmc) or at a point where the leaves hung vertically at the petiole thereby displaying signs of water stress. The water deficit treatment was applied twice during the experiment. Well-watered plants were maintained at 24% vmc. Leaves of the parent clone did not produce any lesions whether under well-watered or water deficit conditions. Line 26-7 produced stippling (lesions) under both treatments, but on less than 0.5% of the leaf surface. Line 18-3 produced significant stippling on both treatments, with almost twice as much stippling under water deficit as under well-watered conditions. Although foliar necrosis was significant for line 18-3, no reduction in growth was apparent.

## POSTER 42

### **A POPLAR PHENYLCOUMARAN BENZYLIC ETHER REDUCTASE IS PREFERENTIALLY EXPRESSED IN LIGNIFYING CELLS AND MAY BE INVOLVED IN DEFENCE AGAINST OXIDATIVE STRESS**

K. Vander Mijnsbrugge, K. Morreel, M. Davey, R. de Rycke, B. Burggraeve, M. Van Montagu, G. Bauw, H. Beeckman, G. Engler, E. Messens and W. Boerjan

Plant Genetics, University of Ghent (KVDM, KM, MD, RDR, BB, MVM, GB, GE, EM, WB) and Wood Biology, Royal Museum for Central Afrika (HB)

krmij@gengenp.rug.ac.be

Lignans constitute a widely distributed class of plant secondary metabolites whose structure is determined by the union of two C6-C3 phenylpropanoids. Still, little is known about their biological significance. One of the most abundant proteins in two-dimensional gels of poplar xylem was shown to be a phenylcoumaran benzylic ether reductase (PCBER). The PCBER from poplar shows sequence homology to isoflavone reductase (IFR), an enzyme involved in the biosynthesis of isoflavonoids, and to (+)pinoresinol/(+)lariciresinol reductase (PLR), an enzyme involved in lignan biosynthesis. IFR homologues have been isolated from several species, under various conditions. Their function is not yet known. The poplar PCBER is strongly associated with lignifying cells. (i) RNA gel blot analysis and protein analysis (immunoblot and immunolocalisations) both confirmed the preferential expression in lignifying tissues and cells. (ii) PCBER is up-regulated in the pith of bent stems, where lignification is induced. In addition, several results indicated that PCBER may play a role in defence against oxidative stress. (i) PCBER is induced upon sulfur starvation (a condition that results in glutathione deprivation). (ii) Over-expression of PCBER in YAP1 mutant yeast complements for stress caused by the thiol-oxidising drug diamide (diamide oxidises glutathione). (iii) Transgenic poplars down-regulated for PCBER show elevated levels of glutathione, both in reduced and oxidised form. From these data, we propose a role for PCBER in defence against oxidative stress, occurring typically in lignifying tissues. Recently several peaks in HPLC chromatograms appeared to be up-regulated in transgenic poplars down-regulated for PCBER. Unravelling their structure may shed new light on the biological role of PCBER activity.

## POSTER 43

### EXPRESSION OF A PINE CYTOSOLIC GLUTAMINE SYNTHETASE GENE IN TRANSGENIC POPLAR

E.G. Kirby, D. Wu, J. Fu, F. Gallardo, and F.M. Cánovas

Department of Biological Sciences, Rutgers University and Department of Biochemistry and Molecular Biology, USA, and University of Málaga

ekirby@andromeda.rutgers.edu  
<http://biology-newark.rutgers.edu/>

We previously reported that a pine cytosolic glutamine synthetase (GS1) cDNA driven by the CaMV 35S promoter has been successfully transformed into hybrid poplar (INRA 7171-B4, *Populus tremula* x *P. alba*) via *Agrobacterium tumefaciens*. Ectopic expression of the pine GS1 lead to increases in GS activity, total soluble protein levels, and chlorophyll contents in leaves. In the current work coupled reverse transcription and PCR amplification (RT-PCR) using pine GS-specific primers was used to assess expression of the transgene message in leaves, stems, and roots of transgenic lines. PCR product was not detected for untransformed poplar tissue, whereas product of predicted size (255 bp) was observed for all transgenic tissues examined, with roots showing the highest levels of expression. Furthermore, RT-PCR using poplar GS-specific primers revealed no co-suppression of endogenous glutamine synthetase expression in transgenic plants. Significant levels of expression of pine mRNA were observed in endodermis of transgenic poplar roots using in situ hybridization, indicating that the nominally constitutive 35S promoter appears expressed in a tissue-specific manner in transgenic poplar plants. An RT-PCR cloning strategy was employed to characterize the poplar GS gene family. A cDNA clone of the cytosolic GS1 has been isolated consisting of 1557 bp and an open reading frame of 1068 bp. The ORF is flanked by untranslated regions of 113 bp at the 5'-end and 373 bp at the 3'-end.

## POSTER 44

### ISOLATION AND CHARACTERIZATION OF A PUTATIVE DEHYDRIN GENE FROM WHITE SPRUCE INDUCED UPON WOUNDING, DROUGHT AND COLD STRESSES

S. Richard, C. Drevet, L. Jouanin, and A. Séguin

Université Laval , Canada (SR, AS), Institut National de la Recherche Agronomique, France (CD, LJ) and Centre de foresterie des Laurentides, Canada (AS)

seguin@cfl.forestry.ca  
<http://www.cfl.forestry.ca>

A cDNA clone encoding a putative dehydrin was isolated from a cDNA library prepared from white spruce needle mRNAs. The cDNA, designated PgDhn1 (for *Picea glauca* dehydrin), is 1159 nucleotides long and has an open reading frame of 735 bp with a deduced amino acid sequence of 245 residues. The putative PgDhn1 amino acid sequence is highly hydrophilic and possesses four conserved repeats of the characterized lysine rich K-segment (EKKGIMDKIKEKLP), and an 8-serine residue stretch prior to the first lysine-rich repeat that is common to many dehydrins. However, the DEYGNP conserved motif is absent in the putative PgDhn1 sequence. The mRNAs corresponding to PgDhn1 cDNA were induced upon wounding, drought and cold, treatment of white spruce seedlings. They were also induced by jasmonic acid (JA) and methyl jasmonate (MeJ). In non-stressed plants, the highest level of transcripts was detected in stem tissue, certain stage of development from male and vegetative buds and in roots to a lesser extent. Transcripts level in needles and female buds was low, and an increase in mRNAs was observed in developing vegetative buds as well as in developing male buds. Following drought stress, a high level of transcripts was observed first in root tissue after 6 hours of treatment. A high level was reached in needle tissue after 2 days of drought stress. Cold-induction of PgDhn1 transcripts was observed in 2-week-old seedlings after 6 days of treatment.

## POSTER 45

### ATTEMPTS TO TRANSFORM HYBRID LARCH WITH A BACILLUS THURINGENSIS GENE

D. Ewald, L. Li, M. Li and H. Yifan

Federal Research Centre for Forestry and Forest Products, Institute for Forest Genetics and Forest Tree Breeding, Germany (DE) and Chinese Academy of Forestry, Molecular Genetics Laboratory, China

ewald@aixh0001.holz.uni-hamburg.de  
<http://www.dainet.de/bfh/e-ins2.htm>

Larch trees are often attacked by insects which can severely damage whole plants. Experiments were carried out to test the possibility to enhance the resistance of this conifer species. A second aim is the use of such plants for risk assessment studies concerning the stability and behaviour of these genetically modified plants. The immature zygotic embryos for the induction of embryogenic lines were harvested from cones of controlled crossings in 1996. This combination of hybrid larch (*L. decidua* x *L. kaempferi*) has shown its outstanding performance in former field trials. A gene from *Bacillus thuringensis* (pBinBt) was used to transform the lines by particle gun. This gene was about 1.8 kb large and a part of the whole gene which was shown to be active in feeding tests after transforming poplar with the same construct. The transformation was carried out by particle gun. Approximately 250 somatic embryos appeared after about 42 days. The somatic embryos were transferred to solid MS medium (+ 412.5 mg/L of NH<sub>4</sub>CO<sub>3</sub>) for germination. One hundred plants were obtained in red light conditions, ninety-four plants were planted into vermiculite. They were planted into the nursery in May 1998. Approximately 30 larger plants were checked by PCR to see if transformation was successful. Fifteen plants showed a positive PCR-reaction. Transgenic larch plants and control plants have been replanted into greenhouse to get sufficient amounts of material for Southern analysis.



## **POSTER 46**

### **FIELD RELEASE OF ROLC-TRANSGENIC ASPEN-POPULUS**

M. Fladung, O. Nowitzki, D. Ebbinghaus, A. Schellhorn, G. Bentien, M.R. Ahuja, and H.-J. Muhs

BFH, Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, Germany

mfladung@rrz.uni-hamburg.de  
<http://www.dainet.de/bfh>

In 1996, the first release experiment with genetically transformed trees in Germany was initiated by our institute in Grosshansdorf. Three different aspen clones were transformed with the 35S-ROLC (Esch5, Brauna11, W52) or rbcS-ROLC constructs (Esch5), and in total eight transgenic and three control lines were planted out on a field with approximately 1500 qm in size. The aim of this experiment is to investigate the stability and expression of foreign genes in transgenic trees on a long term basis as well as under environmental conditions. The transgenic plants are investigated using molecular methods to obtain on a genomic basis generally accepted assessments on stability/instability of foreign genes in transgenic trees. Phenotypical analysis as well as measurements of plant height, stem thickness and leaf-related parameters of the transgenic plants under field conditions revealed constitutive expression of ROLC-related parameters over a period of three years so far. In addition, analyses of the mycorrhizal status and leaf-related phytopathogenic fungi as well as experiments on horizontal gene transfer are currently performed. Differences between clones were found in plant height, leaf parameters and mycorrhiza status. Flushing of buds was also recorded. As it was already shown for greenhouse grown plants, 35S-ROLC plants flushed earlier than their respective controls. The value of this finding is that it is possible to alter the date of flushing by gene transfer methods. At present, a c-DNA library of early-flushed buds of a 35S-ROLC transgenic is constructed to identify genes which are possibly involved in early processes of bud flushing.

## POSTER 47

### MYCORRHIZAL STATUS OF TRANSGENIC POPULUS IN A FIELD TRIAL

M. Fladung, M. Kaldorf, H.-J. Muhs, and F. Buscot

BFH, Institute for Forest genetics and Forest Tree Breeding, Grosshansdorf, Germany

mfladung@rrz.uni-hamburg.de  
<http://www.dainet.de/bfh>

Mycorrhizal symbioses are found in almost all forest trees for which they provide the majority of water and mineral nutrients. An extensive development of mycorrhiza is therefore essential for plant growth under natural conditions and trees modified with biotechnological methods will only be beneficial if both the formation and function of the symbiosis are not affected. The colonization of roots with mycorrhizal fungi is currently investigated in a release experiment with genetically transformed *Populus* clones (see also Fladung and Muhs 1999). The distribution of both ectomycorrhiza (EM) and arbuscular mycorrhiza (AM) is quantified in transgenic and in control plants. Additionally, the diversity of the mycorrhizal populations is analysed using anatomical and molecular markers to characterize EM morphotypes. So far, AM was rare in all root samples investigated. Fully developed EM were found in all probes, and no significant differences in the degree of mycorrhizal colonization were observed for the different *Populus* clones. Even the diversity of mycorrhizal populations is similar in transgenic and in control trees with an average of five different EM fungi found in each root sample. Significant differences were found in the abundance and development of one EM-morphotype which was rare and not well developed on roots from one transgenic *Populus* clone compared to controls. The fungal partner of this EM-type was isolated and will be used in a model system to characterize the interactions between the EM fungus and transgenic trees, respective control trees.

## POSTER 48

### EVIDENCE FOR THE EVOLUTION OF HIGH RATES OF SELFING IN NATIVE POPULATIONS OF *A. MANGIUM* -IMPLICATIONS FOR DOMESTICATION

P.A. Butcher, K. Swann, and G.F. Moran

CSIRO Forestry and Forest Products, Australia

[gavin.moran@ffp.csiro.au](mailto:gavin.moran@ffp.csiro.au)  
<http://www.ffp.csiro.au/tigr/>

*Acacia mangium* is the main commercial species being established in south east Asia for pulp and paper production. As part of ongoing work investigating the genetic resources of *A. mangium*, the level of inbreeding in natural population and seed production stands was determined. Previous studies of RFLP variation had shown higher levels of genetic diversity in populations from New Guinea and Cape York (Queensland) than in populations from the Daintree-Townsville region (Queensland). These differences corresponded with differences in the performance of populations in plantations. Microsatellite markers were used to compare mating system parameters among natural populations in New Guinea and Daintree and a seed production stand in Sumatra. The outcrossing rates in the seed production stand reflected that of the source populations in the Daintree region, suggesting strong genetic control. Trees from the Daintree population had approximately three times the level of selfing of the New Guinea population. Results suggest selection for selfing following past genetic bottlenecks in the smaller and more isolated populations in the south of the species range. The survival of populations in the Daintree region despite high levels of inbreeding is consistent with the historical purging of deleterious alleles and opens opportunities for the use of inbreeding as a tool in breeding programs.

**EUCALYPT PLANTATIONS AND THE SIGNIFICANCE OF LONG DISTANCE POLLINATORS.**

S.G. Southerton, P. Birt and H.A. Ford.

CSIRO Division of Forestry and Forest Products, Australia (SGS) Queensland Parks and Wildlife, Department of Environment, Australia (PB) and University of New England, Department of Zoology, USA (HAF).

simon.southerton@ffp.csiro.au  
<http://www.ffp.csiro.au/>

The arrival of forest biotechnology comes at a time of significant change in eucalypt forestry in Australia. In response to increasing government, commercial and environmental pressures, Australian eucalypt plantations are rapidly expanding. Since eucalypt plantations in Australia are likely to be adjacent to inter-fertile native trees, movement of genes from plantations to surrounding native forests is inevitable, sparking concerns among ecologists. The prospect of transgenic plantations adjacent to native forests has focussed attention on the issue of gene movement. The main vehicle of eucalypt gene movement is pollen. Numerous vectors are attracted to the flowers, which produce abundant nectar and pollen. Most visitors to flowers are insects, including numerous species of beetle, flies and native and introduced bees. Gene movements by these vectors are likely to be limited to relatively short distances from the donor tree. Some features of eucalypt reproductive biology may favour transfer of genes over considerable distances, including outcrossing preference, poor survival of selfs, and prolonged pollen viability. Low frequency movement of eucalypt pollen over distances of several kilometers is not well understood, but may have a significant influence on the genetic makeup of eucalypt forests. The most likely vectors of eucalypt pollen transfer over these distances are lorikeets, honeyeaters and flying foxes, which are widely distributed throughout the range of eucalypt forests. We discuss the potential for these vectors to move eucalypt genes over long distances, and the implications of this for plantation forestry and transgene containment.

## POSTER 50

### RAPD ANALYSIS REVEALS SLIGHT GENETIC DIFFERENTIATION AMONG ECOTYPES OF *AVICENNIA MARINA* FROM KENYA

P.D. Abeysinghe, L. Triest, and N. Koedam

General Botany and Nature Management, Vrije Universiteit Brussel, Belgium

pushpa@vub.ac.be

The genetic variation of populations of *Avicennia marina* from the landward and seaward fringe of the mangrove forest in Gazi bay and Mida creek, Kenya was studied using RAPD (Randomly Amplified Polymorphic DNA analysis). Thirty six RAPD markers were obtained with 12 selected primers for both the landward and the seaward populations of *A. MARINA*. Out of them, A10c allowed to differentiate seaward population (90%) while A12c, J12a and J17d (60%, 96% and 82% respectively) alleles were more frequent for landward populations. PCO (Principal Coordinate Analysis) showed that the seaward population of *A. MARINA* from Gazi bay was separated from the other two landward populations originating from Mida and Gazi (150 km apart) into 2 ecological groups along the second axis which represent 17% of the total variation. The disjunct occurrences can be observed on the cluster analysis as well. Although the landward and seaward populations of Gazi showed extensive morphological variations between populations, this study showed that out of 36 RAPD markers, only 5 markers (~10%) enable us to differentiate slightly the two populations. Amplification of *adh* (alcohol dehydrogenase) gene(s) of *A. marina* using *adh* universal primers showed 4 different bands suggesting that *A. marina* may have 4 *adh* genes. Isozyme analysis of the same plant also show 4 different isozymes.

## POSTER 51

### A CHLOROPLAST-DNA MUTATIONAL 'HOT-SPOT' IN SILVER FIR (*ABIES ALBA*)

B. Ziegenhagen, S. Liepelt, V. Kuhlenkamp, R.J. Petit, and G.G. Vendramin

Institute for Forest Genetics and Forest Tree Breeding (BFH), Grosshansdorf, Germany (B.Z., S.L., V.K.), Laboratoire de Génétique et Amélioration des Arbres Forestières (INRA), Gazinet, France (R.J.P.), Istituto Miglioramento Genetico Piante Forestali (CNR), Firenze, Italy (G.G.V.)

vendramin@imgpf.fi.cnr.it

In the European conifer species silver fir *Abies alba* (Mill.), two polymorphic chloroplast microsatellite loci were detected. Inheritance analysis was performed on the basis of four different controlled crosses including a total progeny of 75 off-springs. The results revealed a predominantly uniparentally paternal transmission from the parental to the filial (F1) generations. Seventeen silver fir populations throughout the range of the species were analysed at the two loci. In 714 investigated individuals, 8 and 18 size variants, respectively, were detected which combined in a total of 90 different haplotypes. In comparison with other genera of the *Pinaceae* (Morgante et al. 1997, Echt et al. 1998, Proban et al. 1998, Vendramin et al. 1998 and unpublished), this is an extremely high value due to the combination of 26 variants at only two chloroplast microsatellite loci. The locus which is represented by 18 size variants appears a mutational 'hot-spot' and is being subjected to extensive sequence-analysis. First results reveal a compound/interrupted microsatellite locus where the repeat numbers of three different mononucleotide stretches contribute to the overall size variation at this locus. The results will be discussed in terms of phylogenetic aspects and for properties of this polymorphism as genetic marker in population genetics.

## POSTER 52

### GENETIC DIVERSITY OF BIRCH (*BETULA PENDULA* ROTH) UNDER OXIDATIVE STRESS

M.S. Korhonen, M.K. Karls, R. Pellinen, H. Tuominen, E.T. Palva And J. Kangasjärvi

Department of Biosciences, Division of Genetics and Institute of Biotechnology, University of Helsinki, Finland

minna-sisko.korhonen@helsinki.fi

<http://www.biocenter.helsinki.fi/groups/kangasjarvi/kangasjarvi.html>

Ozone is a widespread air pollutant that contributes to forest decline. Ozone does not seem to act primarily as a directly damaging agent, instead it causes formation of activated oxygen species (AOS), an oxidative burst in the apoplast that provokes similar processes that occur during the hypersensitive response (HR) in the incompatible plant-pathogen interactions. Plants display a substantial genetic variation in response to both ozone and pathogen attack. To understand the underlying molecular mechanism, we have studied genetic variation in response to ozone stress and compared this to pathogen stress in silver birch. 17 different birch clones, which originate from diverse climatic conditions in Finland were chosen for this study. When exposed to ozone (8-hour pulse, 150 ppb) eight of the clones showed severe damage (more than 20 % damaged leaves). Ozone sensitivity of the different clones correlated with ethylene emission as has been shown in other plant species. At 10 hours hydrogen peroxide was detected in cell walls between palisade parenchyma cells and upper epidermal layer in transmission electron microscope. In order to compare ozone- and pathogen induced responses clones were infiltrated with a non-host pathogen *Pseudomonas syringae* pv *syringae* J900. Infection induced phenotypically diverse HR in different birch clones. We have isolated known stress responsive genes from birch (e.g. genes encoding pathogenesis related proteins and antioxidative enzymes), which will be used to elucidate the genetic variation in response to ozone and pathogen stress in birch.

## POSTER 53

### TISSUE SPECIFIC TRANSGENE EXPRESSION IN APPLE AS A MODEL TREE SPECIES

T.K.Pellny, J.R.Gittins, S.Biricolti, A.J.Passey, and D.J.James

Plant Breeding and Biotechnology, Horticulture Research International - East Malling, UK (TKP, JRG, AJP, DJJ) and Dipartimento di Ortoflorofruitticoltura, Università degli Studi di Firenze, Italy (SB)

till.pellny@hri.ac.uk

<http://www.hri.ac.uk/site2/research/fres.htm>

Restricting expression of transgenes in genetically improved crops to tissues requiring the encoded activity is highly desirable. To this end, the ability of several heterologous gene promoters to drive expression of the beta-glucuronidase (*gusA*) marker gene in the vegetative tissues of a transgenic tree species, the domestic apple (*MALUS PUMILA*, Mill.) has been tested. These promoters originally drove expression in leaves [(Rubisco) small subunit (SSU), RBCS3CP (tomato) and SRS1P (soybean)], vascular tissue (*rolCP* and *CoYMVP*) and root tissue [*extAP* (BRASSICA) and *PsmTaP* (PISUM)]. Transgenic lines (cv. Greensleeves) were produced by AGROBACTERIUM-mediated transformation and the level of *gusA* expression in the vegetative tissues of young plants was compared with that using the CaMV 35S promoter. Replicate transgenic plants (>70 independently derived clones = >300 plants) were propagated in soil to a uniform size and samples of leaf, petiole, stem and root were taken for the fluorometric assay of GUS activity. The precise location of GUS activity was identified at the cell level by staining tissue sections with the chromogenic substrate X-Glucuronide. With the Rubisco SSU promoters, expression was restricted to chlorophyll-containing leaf cells. This expression was light-regulated with the SRS1 promoter but apparently not so with the RBCS3C promoter. The *rolCP* and *CoYMV* promoters produced low level vascular-specific expression. Of particular interest with regard to forestry was the recording of high transgene expression levels in stem vascular tissues, produced by the *extA* and *PsmTA* promoters. In some cases the level of expression exceeded that produced by the constitutive CaMV35S promoter.



## POSTER 54

### ANALYSIS OF MORPHOLOGICAL MUTANTS IN PICEA

M. Fladung, A. Tusch, T. Markussen, and B. Ziegenhagen

BFH, Institute for Forest genetics and Forest Tree Breeding, Grosshansdorf, Germany

mfladung@rrz.uni-hamburg.de  
<http://www.dainet.de/bfh>

Forest trees are long-lived and characterised by long reproduction cycles. Genetic variations which occur at the zygotic stage result in mutant plants, of which rare cases are visible through an altered phenotype. Trees showing such morphologically visible mutations are very often weak in growth. Here, we describe two examples of morphological mutants which will be used for genetic and molecular analysis. The *Picea glauca* 'conica' mutant belongs to the 'dwarf-mutants' and shows a conus-like phenotype. It is a popular ornamental form found in many gardens distributed all over the world. Interestingly, reports are published describing frequently observed reversion in this mutant. These reversion are characterised by a wildtype phenotype. To investigate the genetic background of the reversion, needles from four mutant and reverted branches were harvested and analysed using AFLP-technique. No male or female flowers have been detected in this mutant, since 1998 in one plant cones were detected in the reverted part. After sowing the seeds, a F1-population of approximately 900 seedlings was obtained. The *Picea abies* 'acrocona' mutant is also showing a dwarfed phenotype. Plants derived from open-pollinated progenies revealed an restricted growth habit. It was conducted that the mutation is dominant and, possibly, has occurred in a homeotic gene. In 1993 it was possible, for the first time, to self-pollinate mutant trees and to obtain fertile seeds. Germination of the seeds in 1995 yielded in total 133 seedlings. In 1998 four and 1999 even more seedlings of these only three or four years old plants formed female flowers.

## POSTER 55

### IDENTIFICATION OF PORTUGUESE CASTANEA SPP. CLONES USING RAPD

A.Campilho, I. Santos, and R. Salema

Department of Botany and Institute of Molecular and Cell Biology, University of Porto

anacamp@gaia.ibmc.up.pt

European chestnut (*Castanea sativa*) is a very important species, mainly related to wood and fruit production. In Europe, most of the trees have disappeared due to diseases induced by the fungi of the genus *Phytophthora* and *Cryphonectria*. In Portugal, the area occupied by chestnut trees has decreased to half in the last four decades. To expand the chestnut plantation area, selection of trees tolerant to these diseases is essential and in our country selection programmes started during the forties. Several chestnut clones, which were obtained from crosses between European and Asian chestnuts (*C. mollissima* and *C. crenata*), were produced. Unfortunately, most of the information about their genetic background was lost. Therefore, for the identification of Portuguese chestnut clones, random amplified polymorphic DNA (RAPD) technique was used. This technique is based on the use of short primers of arbitrary sequence in the PCR. Due to sensitivity of the RAPD technique to experimental variables, a suitable protocol, for DNA isolation and for conditions in the PCR, was established. Sixty ten-base-oligonucleotide primers from kit A, E and H (Operon Technologies) were used. The amplification products were separated in agarose gels and UV-visualized with ethidium bromide. From the 60 primers used, 24 generated polymorphisms that were reliably scored. Based on these, Nei's similarity coefficient values were calculated. Cluster analysis (UPMGA) of the similarity indices was conducted to generate dendograms, using NTSYS software. With RAPD technique, some Portuguese chestnut clones were identified and a first evaluation of their relationships was achieved.

## POSTER 56

### DNA EXTRACTION AND PURIFICATION OF MAYTENUS ILICIFOLIA (CELASTRACEAE)

J.V.M. Bittencourt, M.C. Mazza, M. Quoirin

Botany and Phytotechny, University of Parana, Brazil

quoirin@bio.ufpr.br

*Maytenus ilicifolia* (celastraceae) is a shrub of natural occurrence in the Araucaria Forest . It has therapeutic effects for gastric illness. The fragmentation of its habitat and the confirmation as its phytotherapeutic effect has provoked great devastation of its populations. Using techniques of molecular biology it is possible to generate efficient information of the structure and diversity, studying the remaining accesses of *M. ilicifolia* for by chestnut trees has decreased to half in the last four decades. To expand the chestnut plantation area, selection of trees tolerant to these diseases is essential and in our country selection programmes started during the forties. Several chestnut clones, which were obtained from crosses between European and Asian chestnuts (C.MOLLISSIMA and C.CRENATA), were produced. Unfortunately, most of the information about their genetic background was lost. Therefore, for the identification of Portuguese chestnut cloform to remove soluble proteins and carbohydrates in its majority. The precipitation of DNA was obtained by the addition of ethanol and isopropanol in two stages. The results obtained until that moment allow to trace observations addressing more experiments, in order to improve the purification stage, as the accomplished evaluations demonstrated the interference of pollutants, being composed of carbohydrates. The quality of the DNA was determined using agarose gel eletrophoresis stained with ethidium bromide. The adjusted methodology opens the perspective of analysis in the existing populations contributing to the establishment of collection strategies of medicinal germplasm.

## POSTER 57

### CONSERVATION OF GENETIC DIVERSITY OF NORWAY MAPLE IN FINLAND

M. Rusanen

Finnish Forest Research Institute

mari.rusanen@metla.fi  
<http://www.metla.fi/pp/MRus/>

Norway maple (*Acer platanoides*) is growing in Finland in the northern margin of the species' distribution area. Typically it is growing scattered, mixed with other broad-leaved species and spruce, sometimes forming small, fragmented stands. It is one of the few insect-pollinated tree species in Finland and therefore presumed to be inclined towards limited migration and possibly genetic drift. The genetic conservation program for Norway maple is based on an ex situ-approach although two in situ stands (gene reserve forests) have been selected. Research on the population genetic structure was started simultaneously with the gene conservation actions so as to create genetically and economically optimal sampling strategy. Isozyme analyses with 14 loci were done in 26 populations covering the whole distribution area in Finland. The size of the populations ranged from 10 to 300 mature trees. In general, the species did not seem to be deprived of variability; at species level the proportion of polymorphic loci was 50% and the expected heterozygosity was 0.15. Genetic diversity within the populations (expected heterozygosity) ranged from 0.04 to 0.21. No significant correlation was found between the population size and the amount of heterozygosity, neither did any of the populations deviate from Hardy-Weinberg stability. The genetic distances and the geographic distances did not correlate. The implemented conservation strategy of collecting seed from many (30) populations throughout the distribution area and few (10) trees per population, seems to be justified in the light of these results.

## POSTER 58

### RAPD ANALYSIS OF NATURAL POPULATIONS IN CHIMONANTHUS AND ITS SYSTEMATIC VALUE

Longqing Chen, Junyu Chen, Youglian Zhen and Difei Lu

Department of Forestry(LC,DL) and College of Life Science and Technology(YZ), Huazhong Agricultural University; College of Landscape Architecture, Beijing Forestry University, China (JC)

wlichun@sohu.com

Chimonanthus Lindley is an endemic genus in China. The plants of this genus are used for ornamental flowering and pharmaceutical shrubs. Since 1980, its wild resources have been over-exploited and destroyed, and some systematic problems have been also arisen. Thus we conducted the following trials so as to resolve the systematic problems and provide molecular testimony for the conservation of its genetic resources. Leaf samples were collected from 12 natural populations and their DNA were extracted. A total of 323 DNA bands were generated from the DNA samples using RAPD(Randomly Amplified Polymorphic DNA) analysis with 25 arbitrary primers. Based on the DNA bands, the pairwise standard genetic distances among populations and the gene heterozygosity, proportion of polymorphic loci and pairwise genetic distances among individuals within a population, were calculated, and the following conclusions were thus obtained on the basis of the above indices: 1. Meadial dendrogram based on the pairwise standard genetic distances among populations suggested that the genus should be reasonably treated as 4 species, viz *Ch. praecox*, *Ch. campanulatus*, *Ch. salicifolius* and *Ch. nitens*, and genetic relationship between the former two species was closer, the same as that between the latter two species. 2. In 4 populations of *Ch. nitens*, which had been distinguished 2 new species arising argument, existed the greatest genetic variation and differentiation among populations and individuals within a population respectively, as well as the least genetic variation and differentiation among populations of *Ch. praecox* and among individuals within a single population of *Ch. campanulatus*.

## POSTER 59

### STUDY OF THE GENETIC VARIABILITY OF A GOMORTEGA KEULE POPULATION USING ANCHORED PRIMER MICROSATELLITES

R. Herrera, P. Penailillo, M. Arias, and P. Caligari  
Instituto Biología Vegetal y Biotecnología, Universidad de Talca Chile (RH,PP,MA) and  
School of Plant Sciences, The University of Reading, UK (PC)

raherre@pehuenche.otalca.cl

*Gomortega keule* is an endemic tree of the Chilean flora, which is endangered and a unique representative of the Gomortegaceae family. The habitat of the species is severely fragmented and now drastically reduced to only a few specific areas in the coastal region of Central Chile. The homogeneity of the individuals could be the reason for, or could follow, the decrease in the population. Fingerprint analysis from the one population analysed showed that all 15 individuals (representing the whole existing population) had, at least to some extent, distinct DNA patterns. Some anchored primers generated the same fingerprint for a few individuals, but others showed polymorphism to be present. These results suggest that the individuals, from this one population, were the outcome of sexual propagation and are not simply clonal propagules. Thus, this initial study has already demonstrated that molecular marker technology can be used to expose the genetic variability within a *Gomortega keule* population. The present results show that at least the remaining individuals within this population do have different genetic constitutions with some polymorphism being exhibited. Further analyses of other populations, existing in the same general area but separated by 100 km, are needed to determine the wider pattern of variation within and between populations, and for conservation strategies to be determined.

This work was supported by Dirección de Investigación Universidad de Talca, UNESCO-Biotechnology programme and Chilean Academic Links Programme (CONICYT & British Council).

## POSTER 60

### INTROGRESSION OF PICEA RUBENS AND P. MARIANA IN THE MARITIME REGION OF CANADA

L.L. DeVerno and J. Loo

Natural Resources Canada, Canadian Forest Service, Canada

ldeverno@x1.nrcan.gc.ca

Red and black spruce are sympatric in the Canadian Maritime Provinces. Without ecological isolation or reproductive barriers to maintain genetic integrity, these two species will hybridize, threatening the purity of both gene pools. Clearcutting in New Brunswick appears to favor regeneration of either black spruce or hybrids because red spruce is more shade tolerant and seedlings have higher requirements for moisture, resulting in poor regeneration performance. Field identification of red and black spruce can be difficult and the extent of natural hybridization has been controversial. In this study, several monomorphic species-specific RAPD markers were identified in allopatric red and black spruce provenances. These markers were used to evaluate old growth red spruce stands in Nova Scotia and New Brunswick, as well as populations of bog black spruce in New Brunswick. In addition, New Brunswick red and black spruce trees from a previous study, identified on the basis of a number of morphological characteristics, were analyzed with the species-specific RAPD markers. Results indicate that at least some of the mature stands of red and black spruce in the Maritimes contain introgressed individuals, and that morphology is an unreliable method to distinguish among red spruce, black spruce and introgressed individuals. Hybridization and introgression of these two spruce species does not appear to be a recent phenomenon.

## POSTER 61

### GENETIC ANALYSIS OF EUCALYPTUS GRANDIS.

K. Christelle Klopper, Brenda D. Wingfield, Anna-Maria Botha

Dept of Genetics, Forestry and Agricultural Institute, University of Pretoria, Pretoria, 0002, South Africa

ambothao@postino.up.ac.za

*Eucalyptus grandis* is a tall, straight tree, growing rapidly up to 50 metres high under good environmental conditions. In South Africa *E. grandis* is mainly planted for its use in the pulping and mining industries. It is a commonly grown *Eucalyptus* species in South Africa and currently occupies 77% of the area planted to *Eucalyptus*. It is thus, economically important to increase the yield by mass-production of the selected superior genotypes. Recalcitrant mature trees were proliferated by micropropagation. A direct correlation was observed between disease resistance and the production of phenolic compounds. This was inversely correlated with the rooting ability. The short-term objective of this study was to determine the genetic diversity of the 14 clones of a commercially grown *E. grandis* half-sib family. The degree of genetic relatedness and quantification of the genetic diversity in this study was done, using RAPDs, PCR-RFLPs and AFLPs. This has the potential to result in a molecular marker linked to the production of the phenolic compounds, disease resistance or rooting ability of these clones. Marker aided selection; selection of desired traits by molecular markers is a longer-term goal of this study.



## POSTER 62

### GENETIC RELATIONSHIPS AND VARIATION IN THE GENUS *PICEA* ASSESSED BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND CYTOLOGICAL ANALYSES.

K.K. Nkongolo

Department of Biology, Laurentian University, Canada

knkongol@NICKEL.LAURENTIAN.CA

A wide array of techniques have been used in the studies of forest tree relationship and variation. In the genus *Picea* which comprises 40 to 60 species, genetic relationships among species have been mainly determined by crossability and morphological characteristics. Due to a high level of variation, classification of such a large genus has proved difficult and many ambiguities still remain. In the present study, genetic variations within and among *Picea spp.* were investigated using random amplified polymorphic DNA (RAPD). Polymorphism in RAPD markers was sufficient to distinguish each of the species. The degree of band sharing was used to evaluate genetic distance between species and to construct a phylogenetic tree. Species - specific markers were used to identify interspecific hybrids. These markers are being cloned and sequenced to design specific primers. Cytological data also reveal distinctive characteristics for each species analyzed. The spruce karyotypes are generally asymmetrical with smaller chromosomes being more submedian than the larger ones. Point dispersal patterns of diagrammatic presentations of species karyotypes and chromosome structures were found to be useful characteristics in the analysis of the evolution process and species differentiation within the genus *Picea*.

## POSTER 63

### MAPPING GENES OF THE BIOSYNTHETIC PATHWAY OF LIGNINS IN EUCALYPTS.

Jean-Marc Gion, Philippe Rech, Jacqueline Grima-Pettenati, Daniel Verhaegen and Christophe Plomion

Cirad-Forêt , France (J-M.G., P.R., D.V.), UMR CNRS/Université Paul Sabatier, France (J.G-P.), Laboratoire De Genetique Et Amelioration Des Arbres Forestiers , France

gion@cirad.fr

Single-strand conformation polymorphism analysis (SSCP) is a simple technique to map genes of known function. We used this method for mapping 6 genes implicated in the biosynthetic pathway of lignins on *Eucalyptus urophylla* and *Eucalyptus grandis* linkage maps. We localized 4 genes involved in the common phenylpropanoid pathway (COMT, CCoAOMT, 4CL and PAL) and 2 genes involved in the "lignin specific" pathway (CCR and CAD2). The sequencing of PCR products confirmed SSCP results. These genes have been mapped on 4 of the 11 linkage groups constructed by Verhaegen and Plomion (1996). We observed more polymorphism in the *E. urophylla* parent than in the *E. grandis* parent. For 3 genes *E. grandis* appears to be homozygote for the amplified fragment. The locations on the *Eucalyptus* linkage maps suggested that we could expect more allelic combinations in the progeny than in gene clusters. From this genotypic variability we can expect heterogeneity in related traits.

**POSTER 64**

**A VALIDATION EXPERIMENT OF QTL FOR VEGETATIVE PROPAGATION TRAITS IN EUCALYPTUS**

C.M. Marques, V.J. Carocha, and J.G. Ferreira

Raiz - Instituto de Investigacao da Floresta e Papel

crismarq@itqb.unl.pt

It is likely that some QTL are environment and/or genetic background specific. The QTL that are consistently important would be the most useful for marker assisted selection and very interesting candidates for positional cloning. We have detected QTL controlling vegetative propagation traits in an interspecific family (two hundred and one F1 individuals) of *E. tereticornis* and *E. globulus* (Marques et al nineteen ninety nine, Theor Appl Genet, accepted). We report on QTL validation results obtained after genotyping an independent set of eighty seven individuals of the same progeny set.

## POSTER 65

### IDENTIFICATION OF QTL MARKERS FOR WOOD DENSITY OF PICEA

T. Markussen, A. Tusch, B. Karlsson, L. Wilhelmsson, R. Stephan, and M. Fladung

BFH, Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, Germany  
SkogForst, Ekebo, Svalöv, Sweden, SkogForsk, Science Park, Uppsala, Sweden

markusse@aixh0001.holz.uni-hamburg.de  
<http://www.dainet.de/bfh>

Norway spruce (*Picea abies* (L.) Karst.) is one of the most important forest trees of the northern hemisphere. Among the different wood quality traits, wood density is a key property highly correlated with lumber and fiber properties as well as pulp yield. A high and uniform wood density is desirable for many solid wood products, while high or low wood density is favourable depending on the paper product and process considered. Wood density is mainly influenced by fiber geometry and fiber wall thickness. Within a species the variation in chemical composition seems to be less important. The fiber formation is controlled by physiological processes depending on genetic and environmental conditions along cambial ageing. The aim of the project (short title: GENIALITY), supported by the European Commission is the genetic improvement of wood quality and the increase of selection efficiency for different end uses. Within this project an essential part is the identification of QTL-markers for wood density of *Picea abies*, which can be used in early selection tests. The approach for the identification of molecular markers for wood density is following the strategy of bulked segregant analysis (BSA). For the analysis genotypes were selected from Swedish crossing experiments. Individuals with the extremes of high and low wood density were used to construct DNA-pools for the identification of QTL markers via AFLP and RAPD analysis. First results show differences between the two pools, which must be validated in single individuals.

**POSTER 66**

**DETECTION OF POINT MUTATIONS FOR COMPARATIVE MAPPING IN PINACEAE**

S. degli Ivanissevich, M. Morgante

Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, Italy

stefania.ivanissevich@dpvta.uniud.it

The large genome size of the conifers has often made studies of genome expression, structure and mapping difficult. The possibility to construct syntenic genetic maps in conifers would therefore be of great interest. Mapping information and molecular marker data on a single species could be transferred on the other species, avoiding thereby the dispersion of the resources. The main objective of our work is to establish the presence of synteny among different conifer species and discuss its implications for comparative mapping. The goal is to determine the transferability of EST markers across *Pinaceae* to use them in the construction of syntenic genetic maps. c-DNA sequences in Norway spruce will be investigated with the aim to obtain anchor loci for EST mapping. Different techniques will be used to identify polymorphisms. SSCP (Single-stranded DNA conformation polymorphism) and CFLP (Cleavase fragment length polymorphism) techniques seem to give the best results detecting 80%-95% of the point mutations. The polymorphic markers will be mapped onto the PICEA ABIES and PINUS PINASTER mapping populations, for which maps are already existing, in order to gain knowlegde about the syntenic relationships between the two species.

## POSTER 67

### EARLY FLOWER INDUCTION RESULTS FROM ECTOPIC EXPRESSION OF BIRCH MADS BOX GENES BpMADS3, BpMADS4 and BpMADS5

A. Elo, J. Lemmetyinen, K. Keinonen-Mettälä, and T. Sopenen

Department of Biology, University of Joensuu, Finland

Annakaisa.Elo@joensuu.fi

<http://www.joensuu.fi/matemluonto/biologia/sopenen/sopenen.html>

Three MADS box genes BpMADS3, BpMADS4 and BpMADS5 from silver birch (*Betula pendula* Roth) showed high similarity to known floral and inflorescence meristem identity genes like APETALA1, SQUAMOSA and AGL8. According to analyses of RNA expression, all three birch genes were active during the development of both male and female inflorescences. BpMADS3 seemed to have the highest expression in late developmental stages, but also in vegetative shoot tips. BpMADS4 was expressed, in addition to developing inflorescences, also in shoots and in roots. The expression of BpMADS5 seemed to be inflorescence-specific. Ectopic expression of either BpMADS3, BpMADS4 and BpMADS5 with the CaMV 35S promoter in tobacco resulted in early flowering plants. The early flowering tobacco plants were fertile and the phenotype was inherited to progeny as a dominant trait. Overexpression of BpMADS4 in our early flowering test birch lines resulted in plants flowering remarkably earlier and smaller sized (5-10 cm high) than non-transgenic controls. These ectopic expression experiments suggest that these three birch genes play a role in determining the identity of either the inflorescence or the floral meristem. These genes could possibly be utilized in producing early flowering plant lines e.g. for breeding or transgene testing purposes.

## POSTER 68

### MICROSATELLITE FINGERPRINTING OF EUCALYPTUS IN SOUTH AFRICA

B.D. Wingfield, M. van der Nest, F.C. Blakeway and N. Edwards

Dept. Genetics, FMBC and FABI, University of Pretoria (BDW, MvdN), Mondi Forests (FCB, NE) South Africa.

brenda.wingfield@fabi.up.ac.za

*Eucalyptus grandis* and *E. grandis* hybrids are widely utilised in exotic plantations in South Africa. A large number of the eucalypts in these plantations are derived from vegetative propagation. Routine verification of the identity of these clones using molecular markers has become increasingly important to the South African Forestry Companies. This enables the resolution of problems linked to mistaken identity and is also important where clones are being licensed to other groups for propagation. The most widely used technique for molecular fingerprinting at this stage is RAPDs. It is however difficult, to standardise RAPD profiles produced in different laboratories. Recently a number of simple sequence repeats (SSRs) or microsatellites have been developed by the Forest Molecular Biology Co-operative Programme (FMBC) to be used as markers for *Eucalyptus* in plantations. These markers target specific alleles and, therefore, produce data that are more readily comparable between laboratories. The aim of this study was to evaluate SSR markers that are in the public domain as well as those developed in our laboratories. Evaluation has been based on South African *E. grandis* and hybrid material as well as a pure *E. grandis* pedigree, which we have developed for this as well as other studies. All the SSR markers segregated in a typical 1:1 ratio. We were thus able to identify the linkage groups of our markers relative to a previously published *E. grandis* linkage map. This study is part of a larger international initiative aimed at producing fingerprinting markers for *Eucalyptus* and developing saturated linkage maps of the most commonly deployed *Eucalyptus* species.

## POSTER 69

### CHARACTERISATION AND GENOMIC ORGANISATION OF REPETITIVE DNA IN NORWAY SPRUCE

F. Cattonaro, I. Jurman, A. Zuccolo, M. Vizzi, M. Morgante

Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, Italy

federica.cattonaro@dpvta.uniud.it

Alternative strategies for library construction were used in order to explore the whole range of repetitive sequences in Norway spruce. Total genomic DNA was used to screen small insert genomic libraries by dot blot hybridisation. Overall a large portion of the clones were represented by long interspersed repeats as copia- and gypsy-like retrotransposons, while SINE-like elements were rare. Tandem repeats (satellite DNA, microsatellite) did not seem to be very abundant in the genome; MITEs (Miniature Inverted-Repeat Transposable Elements) and LINEs (Long Interspersed Nuclear Elements) were not represented. Thirty-four clones of all the major families identified were used as probes on genomic Southern blots to obtain information about the organisation pattern, the dispersal and the methylation state. Two genomic libraries were constructed using the hypermethylated fraction (low molecular weight) and the hypomethylated fraction (high molecular weight) of the digest with McrBC enzyme. The hypermethylated fraction was largely composed by repetitive sequences, while the hypomethylated library was highly enriched in low copy number sequences, which are likely to represent gene-rich sequences. Methylation degree of different repeats type was investigated by comparing the hybridisation pattern of DNA digests using McrBC enzyme, HpaII, MspI and EcoRI as control. In order to establish the level of repetitive sequences conservation among different Pinaceae families, 87 of the 120 repetitive sequences isolated from Norway spruce, representative of the major group identified, were spotted on dot blot. The blot was hybridised with total labelled genomic DNA from different Pinaceae species.



## POSTER 70

### PORTUGUESE BREEDING PROGRAM OF MARITIME PINE SUPPORTED BY MOLECULAR BIOLOGY

P. Costa, M Rocheta, A. Regalado, A. Ribeiro, M.J. Rijo, C. Miguel, C. Pinto Ricardo and P. Fevereiro

Instituto de Biologia Experimental e Tecnológica, Portugal

pauloc@itqb.unl.pt

Maritime pine is the most important conifer in Portugal. However, actual pine forest production is not enough to provide wood industry. Genetic improvement allows increasing yield, quality and adaptability of the specie. In order to support the actual breeding program of maritime pine in Portugal with new biotechnology tools, the team *Pinus* was created in IBET in November 1998. The activity of this group concerns essentially vegetative propagation (see poster *in vitro* propagation of maritime pine (*Pinus pinaster*) for added-value forestry) and the use of biomolecular tools to support selection. Nowadays, the diversity of the breeding population is being evaluated with RAPD, AFLP and MP-PCR markers. Those analysis and comparison between information provided by each kind of marker will be discussed. In order to obtain markers of economical interest, gene discovery projects are also in course. The study of differentially expressed genes in roots and needles of maritime pine young seedlings submitted to different nutrition and water supplies will allow the identification of genes involved in stress responses. These studies are being applied to different genetic backgrounds. Gene polymorphisms will be than analysed in the breeding population in order to assist selection for adaptation of the species in Portugal.

## POSTER 71

### USE OF DIFFERENTIAL DISPLAY TO IDENTIFY ALNUS GLUTINOSA GENES INVOLVED IN THE INITIATION AND DEVELOPMENT OF A SYMBIOTIC LATERAL ROOT

L. Laplaze, R. Heidstra, C. Franche, E. Duhoux, D. Bogusz, and K. Pawlowski

Physiologie Cellulaire et Moleculaire des Arbres, IRD-GeneTrop, France

franche@mpl.ird.fr

The interaction between the soil actinomycete *Frankia* and actinorhizal plants, leads to the formation of nitrogen-fixing root-nodules. Whereas legume root nodules have a stem-like structure, actinorhizal nodules are modified lateral roots, i.e. they have a central vascular bundle and they arise from cell division in the pericycle. ALNUS infection starts with the induction of root hair curling by an unknown *Frankia* signal. Hyphae penetrate deformed root hairs and limited cell divisions are induced in the root cortex near the invaded root hair, giving rise to a swollen region, the so-called prenodule. Infection threads, consisting of lines of encapsulated hyphae, progress intracellularly toward this mitotically active zone and then further invade some cells of the prenodule. In the same time, divisions start in the pericycle opposite a protoxylem pole, leading to the formation of the prenodule primordium. The nodule primordium grows and cortical cells are infected by *Frankia* hyphae coming from the prenodule. The aim of this project was to understand the molecular events associated with this lateral root development. In order to find the genes involved in nodule initiation and development, we used differential display to compare ALNUS GLUTINOSA uninfected root and prenodule mRNA populations. The differential analysis was conducted using different types of anchor primers yielding 41 prenodule-specific bands. The characterization of the corresponding products is in progress.

**POSTER 72**

**SOMACLONAL VARIATION IN CRYOPRESERVED EMBRYOGENIC CLONES OF WHITE SPRUCE [PICEA GLAUCA (MOENCH) VOSS.]**

L.L. DeVerno, Y.S. Park, J.M. Bonga, J.D. Barrett

Natural Resources Canada, Canadian Forest Service

ldeverno@x1.nrcan.gc.ca

Trees were regenerated from six white spruce embryogenic clones after cryopreservation for 3 and 4 years. Genetic stability was evaluated using randomly amplified polymorphic DNA (RAPD) fingerprints. Somaclonal variation was detected in some *in vitro* embryogenic cultures 2 and 12 months after they were re-established following cryopreservation, but not in the corresponding regenerated trees. These results suggest that trees regenerated from cryopreserved cultures in subsequent years are primarily genetically stable in the genomic regions tested, and variation observed due to the *in vitro* culture process infrequently affects trees regenerated from normally maturing and germinating somatic embryos. However, trees regenerated from somatic embryos that matured or germinated abnormally in *in vitro* culture exhibited altered RAPD fragment patterns.

## POSTER 73

### IDENTIFICATION OF DROUGHT-STRESS REGULATED GENES IN MARITIME PINE

C. Dubos, J-M. Frigerio, C. Lalanne, D. Madur and C. Plomion

INRA-Forestry Department

dubos@pierroton.inra.fr

<http://www.pierroton.inra.fr/genetics/>

In this study we present a global (EST sequencing) and a targeted (differential screening of mRNA) approach to identify drought-stress regulated genes in root and aerial part of 7 week-old maritime pine seedlings. cDNAs were obtained from stressed and non stressed plants (root and aerial parts). Stress (-0.45 MPa) was applied in hydroponic medium with PEG3350. The global approach is based on the comparison of EST redundancy profiles obtained from stressed and non-stressed plants. As an example, nodulin-like protein, annexin, geranyl-geranyl pyrophosphatase and chitinase were repeatedly sequenced in the cDNA library constructed from stressed roots. A specific group of mRNAs involved in drought stress response was selected using the mRNA-fingerprinting method based on AFLP. 96 primer enzyme combinations (PEC) allowed to amplify cDNA fragments ranging from 50 to 800bp. They were separated by electrophoresis on polyacrylamide gels and silver stained. An average of 50 bands per PEC was obtained in both tissues resulting in a total of 10,000 screened fragments. 300 and 150 cDNAs were found to be differentially expressed in the aerial part and in root, respectively. 30% of these fragments displayed a clear presence:absence variation and were sequenced. Among, differentially expressed genes in the aerial part, Lhca4, flavonoid hydroxylase, acid phosphatase, pathogenesis-related proteins, pectin methylesterase were among the genes that were highly expressed in the stressed medium. Some genes showed similar expression in both approaches: e.g. the ssRubisco was found to be highly expressed in non-stressed seedlings; the Lhca was found to be highly expressed in stressed plants.

## POSTER 74

### ISOLATION OF WOUND-INDUCIBLE GENES FROM CASTANEA SATIVA BY THE DIFFERENTIAL mRNA DISPLAY METHOD

R. Schafleitner, K. Hohl, and E. Wilhelm

Austrian Research Centers Seibersdorf GMBH, Austria

roland.schafleitner@arcs.ac.at

Wounds in plants cause restriction of the evaporation protection and impairment of the water transport. Furthermore, wounds represent entrance opportunities for pathogenic micro-organisms. Our model plant chestnut is endangered by a wound parasite, *Cryphonectria parasitica*. Here, the efficiency of wound closure may influence the disease incidence. Also in the course of the treatment of cankers with hypovirulent *Cryphonectria*, wound healing is a crucial process. To know more on the wound response of plants and to integrate histological and molecular data on wound healing, a search for wound-inducible genes using the differential mRNA display methodology was performed, comparing the gene expression in chestnut *in vitro* shoots three hours after wounding with that of untreated control plants aim at the isolation of genes involved in the very early response. Until now we have identified 3 fragments of wound-inducible genes: Fragment 1 shows homology to the BCY1 gene encoding the regulatory subunit of a cAMP-dependent protein kinase from yeast and to an *Arabidopsis thaliana* phosphatase gene. Fragment 2 exhibits high homology to the *Arabidopsis thaliana perl* (peroxiredoxin) gene and Fragment 3 apparently is homologous to the pectin esterase gene of *Arabidopsis thaliana*. The fragments cloned are positioned at the 3' end of the cDNAs and the stretches conferring homology to other genes are rather small. Thus for the final determination of the identity and function of these wound-inducible genes further investigations will be essential.

## POSTER 75

### FIRST RESULT IN THE GENETIC TRANSFORMATION OF FOUR TROPICAL ACACIA SPECIES: *A. ALBIDA*, *A. CRASSICARPA*, *A. MEARNsii* AND *A. MANGIUM*

M. Quoirin, A. Galiana, D. Goh, A. Limanton, V. Gratio, J. Ahee, M. Rio, D. Oliveira, E. Duhoux & C. Franche

Departamento de Botanica, Universidade Federal do Parana, Brazil (MQ), Plant Tissue Biotechnology Laboratory, INNOPRISE Corporation Sdn Bhd/CIRAD Forjt (AG, DG), Biotechnologie des Symbioses Forestieres Tropicales (AL,VG,JA), Genetics, Universidade Federal do Rio de Janeiro, Brazil (D0), Physiologie Cellulaire et Moleculaire des Arbres, IRD (ex ORSTOM) (MR, ED,CF)

quoirin@bio.ufpr.br

*Acacia albida*, *A. crassicarpa*, *A. mearnsii* and *A. mangium* belong to the Leguminous family. In our experiments, we evaluated two strategies of genetic transformation: the biolistic method and the use of a natural vector, *Agrobacterium tumefaciens*. At the same time, a protocol of shoot regeneration from cotyledons of *A. crassicarpa* was developed and adapted for *A. mangium* and *A. mearnsii*. Particle bombardment technique was applied to various types of explants of *A. crassicarpa*, *A. mangium* and *A. mearnsii*. Tungsten beads covered with plasmid pUC18 (carrying CaMV 35S or e35S promoter, UIDA gene, NOS 34end) were delivered to the explants by a helium apparatus. Transient expression of the reporter gene was observed in all explants. Helium apparatus proved to be superior to the gun powder device in experiments with *A. crassicarpa*. Various parameters of bombardment were evaluated in order to optimize the technique. Stably transformed tissues were not recovered yet. The susceptibility of the four *Acacia* species to wild-type strains of *Agrobacterium tumefaciens* was studied. *A. mangium* appeared as the most susceptible species. Strains capable of naturally inducing shoot differentiation on the tumors were also tested. Some shoots were formed on tumors induced by the strain Antib12 on *A. albida* hypocotyls. Further on, the strains LBA4404 and EHA101 were used. Both contained the plasmid vector pBIN19GUSINT. Cotyledons and hypocotyls of *A. crassicarpa* and *A. mangium*, and two months old seedlings of *A. crassicarpa* were inoculated. Some beta-glucuronidase activity was visible on cotyledons of *A. crassicarpa* 21 days after inoculation.

# **PARTICIPANTS**