UNIVERSITY OF PRETORIA

SECURITY SERVICES

A Safety Guide for our International Visitors

Crime happens anywhere, at anytime, to anyone!

Safety Tips

Do not leave attractive or valuable items near windows, on car seats, or anywhere where they can be seen by passers-by.

When out walking

- Never walk alone, always in groups of 2 or more.
- Avoid the same route every time.
- Tell someone where you are going and how long you will be away.

Always carry your cellphone either on a belt or in a travel wallet around your waist.

Never accept a lift from a stranger.

When shopping, keep your wallet, purse, and credit cards on your person. Be wary when negotiating or examining merchandise. Make sure that you are not being pick-pocketed. Wallets and purses should never be put into the back pockets of trousers.

- Avoid carrying large amounts of money with you.
- Do not flash large amounts of money in public.
- Always limit the number of bags, parcels, or packages that you carry so that you are not overloaded and vulnerable.

Keep valuables locked up.

Keep rooms locked. Do not lend your keys to anyone.

Criminals target easy victims. Do not afford them the opportunity. Show a confident attitude.

Criminals often target tourists whom they identify by different languages or clothing, as they know that court proceedings will take longer than the tourist's holiday –if they are caught!

Crimes that frequently occur:

- Theft of and from motor vehicles
- Theft of cellphones and purses
- Hi-jacking
- Robbery (ATM's)
- Muggings

Criminals of every category rely on opportunity and so it is sensible to reduce that opportunity wherever possible.

We have to stop thinking "It's never going to happen to me" and start thinking "What can I do to prevent it from happening to me?"

Important telephone number

Police (Flying Squad)	-	10111	UP Crisis Line	-	0800 006428
University Security Services (24 hours)	-	(012) 420 2310 083 654 0476	Brooklyn Police Fire Brigade	-	(012) 362 1500 10177 / (012) 342 5979

Any other safety related enquiries

Bes Liebenberg - (012) 420 4344

Welcome, enjoy your stay in South Africa

CONTENTS

LIST OF SPONSORS

We are most grateful to the following sponsors who have contributed so generously towards the success of this IUFRO meeting:

City of Tshwane Metropolitan Municipality – Mayoral Opening Banquet

SAPPI – Gala Farewell Dinner, abstracts book, pens

Mondi Business Paper South Africa – Gala Farewell Dinner, desk pad boxes and MondiBP caps

SweTree Technologies – Conference bags and T-shirts

DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) – Conference centre hire

National Research Foundation (NRF) – Contribution towards keynote speaker costs

Forestal Oriental SA (FOSA) – Lanyards

Tree Protection Co-operative Programme – Group photo

SAA – Special rates on flight tickets

Separation Scientific – Contribution towards conference costs

Forest Molecular Genetics Programme - Contribution towards conference costs – *Eucalyptus* Genome Breakfast

Gauteng Tourism Authority – Beaded photo frames; use of information kiosk at international arrivals hall, Johannesburg Airport

Central Timber Co-operative (CTC) – Contribution towards conference costs

Bio-rad – Keyrings

Tshwane Visitors CC – Visitors' Guide

Roche Molecular Diagnostics – Pens

AEC – Amersham Pty Ltd – Mineral water

CONFERENCE ORGANISATION

Hosted by: University of Pretoria, Pretoria, South Africa
Forestry and Agricultural Biotechnology Institute (FABI)

Organised by: Prof. Brenda Wingfield and Dr. Zander Myburg

Current Working Party Committee (IUFRO Division 2, Unit 2.04.06 Molecular biology of forest trees)
Björn Sundberg (Coordinator, Sweden), Zander Myburg (Deputy, South Africa) and Dave Ellis (Deputy, USA)

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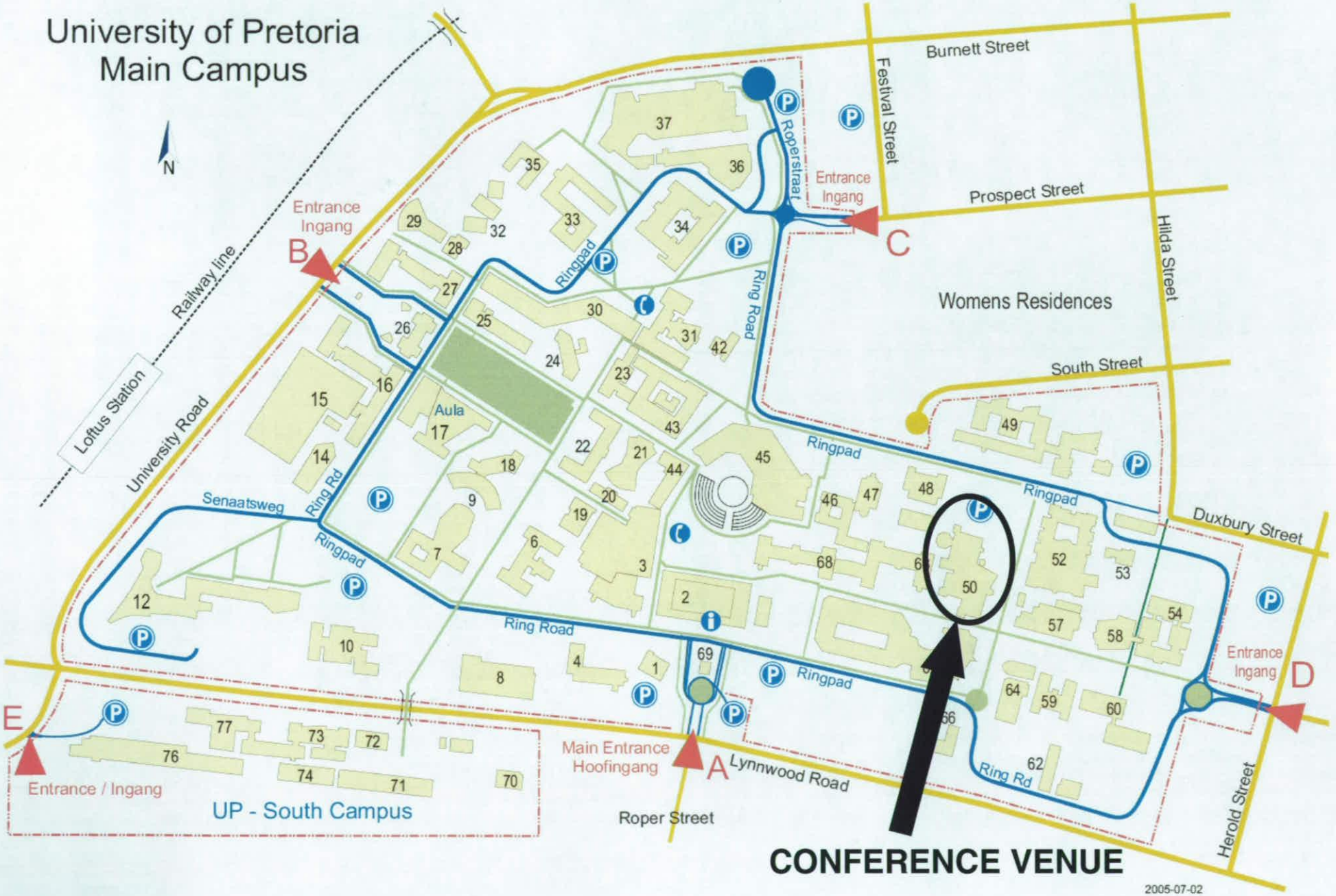
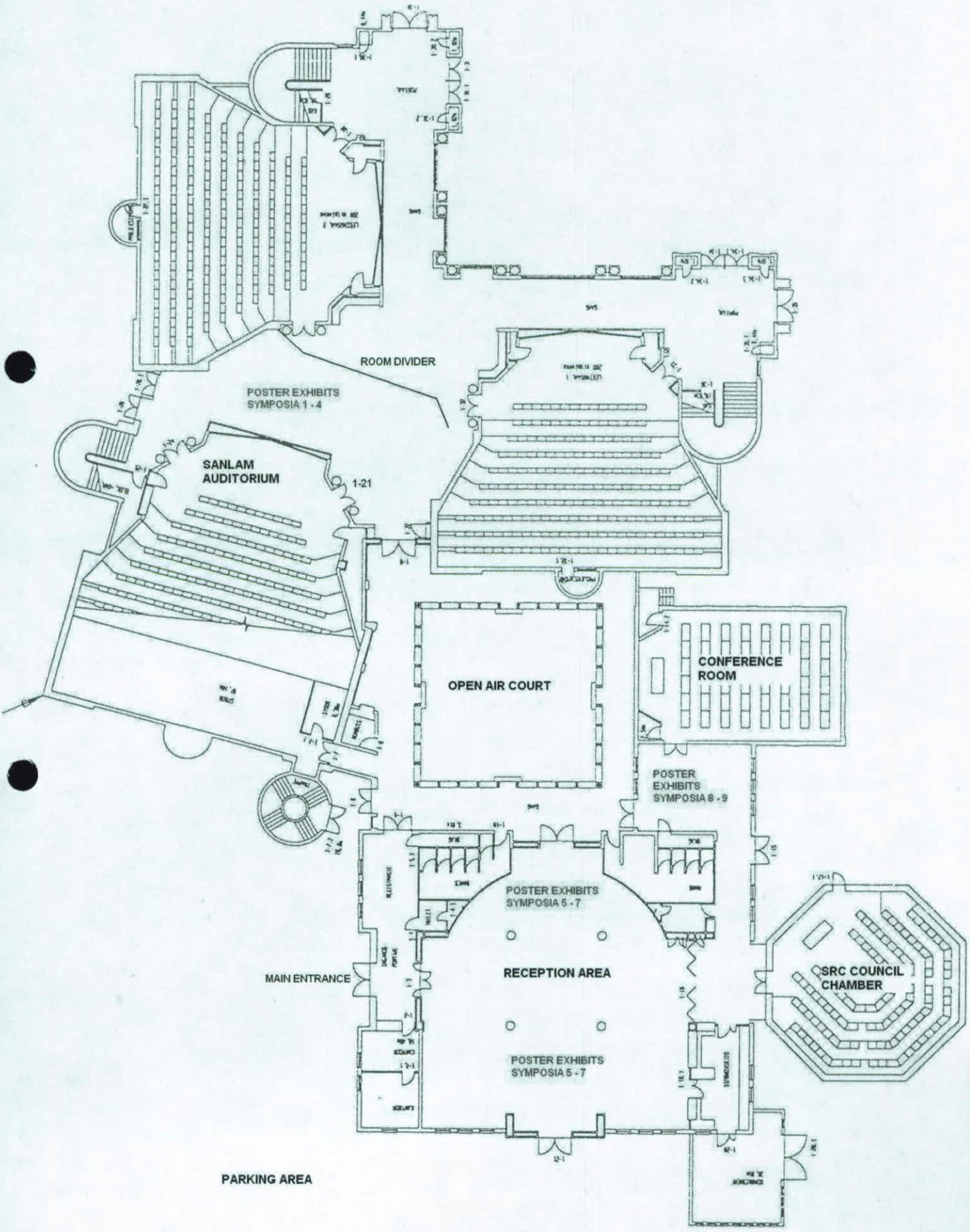
Conference Secretariat
Sarie Mehl, Corrie Moll and Althea Holworthy
(iufro2005@conferencecontacts.co.za, Tel: 083 252 7094)

CONFERENCE VENUE LOCATION



CONFERENCE VENUE FLOOR PLAN AND POSTER LAYOUT



DIRECTIONS TO SAMMY MARKS CONVENTION CENTRE

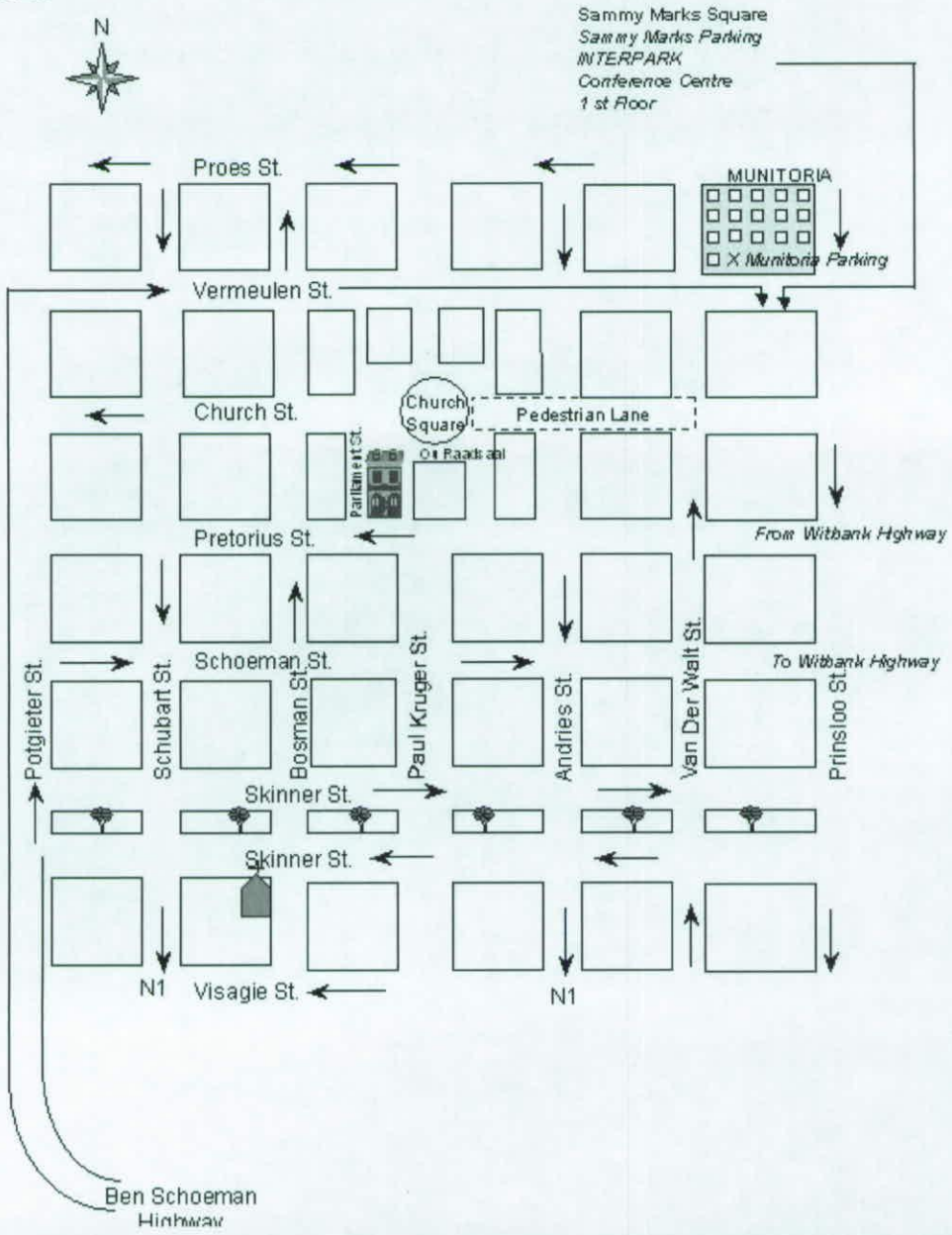
(Monday 7 November 2005)

Tel:
Danie (012) 358 4931
Chantel (012) 358 4775

From the University main gate turn left into Lynnwood Road, turn left into Duncan Street and left into Pretorius Street (one-way).
Turn right into Van der Walt Street (one-way) and right into Vermeulen Street (one-way).

Keep to your right.
On the right hand side (just past the bridge leading to Munitoria), you will see Interpark "Public Parking" and a sign to the Sammy Marks Convention Centre
Turn right into the parking area.

Follow the signs in the parking to the lift.
Select the first floor – there is a Sammy Marks Convention Centre Label next to it.
Follow signage from the lift to the entrance to the Sammy Marks Convention Centre.



BUS SCHEDULE

SUNDAY 6 NOVEMBER
DEPARTURE TIMES FROM HOTELS FOR REGSITRATION

Bus No	1	2	3
SHERATON HOTEL	13:30	15:15	17:00
COURT CLASSIQUE	13:45	15:30	17:15
COURTYARD	14:00	15:45	17:30
HOLIDAY INN HATFIELD	14:15	16:00	17:45
HATFIELD MANOR HOTEL	14:30	16:15	18:00
HATFIELD LODGE	14:45	16:30	18:15

RETURN TO ALL HOTELS AFTER FUNCTION AT 21:30

7 – 11 NOVEMBER 2005.

ROUTE 1 – SHERATON
MORNING:
07:40 Depart Sheraton Hotel
08:00 Arrival University of Pretoria

AFTERNOON
17:15 Departure University of Pretoria
17:25 Arrival Sheraton Hotel

ROUTE 2 – ARCADIA
MORNING:
07:30 Depart Court Classique
07:45 Depart Courtyard Arcadia
08:00 Arrival University of Pretoria

AFTERNOON
17:15 Departure University of Pretoria
17:25 Arrival Courtyard Arcadia
17:35 Arrival Court Classique

ROUTE 3 – HATFIELD
MORNING:
07:30 Depart Holiday Inn Hatfield
07:45 Depart Hatfield Manor Hotel
08:50 Depart Hatfield Lodge
08:00 Arrival University of Pretoria

AFTERNOON
17:15 Departure University of Pretoria
17:25 Arrival Hatfield Lodge
17:30 Arrival Hatfield Manor Hotel
17:40 Arrival Holiday Inn Hatfield

CONFERENCE PROGRAMME OVERVIEW

Sunday – 6 November 2005

14:00 **Conference Secretariat opens**

Conference registration from 14:00 to 18:00

Poster boards available – all posters must be on display by 18:00

18:00 Official welcoming on behalf of the University of Pretoria

18:15 Official welcoming on behalf of FABI and IUFRO (Mike Wingfield)

18:30 Overview of Conference Schedule and General Information (Zander Myburg and Brenda Wingfield)

19:00 Cheese & Wine Cocktail Welcome Reception at Sanlam Auditorium

Monday - 7 November 2005

08:15 Official welcoming on behalf of the IUFRO Working Party (Björn Sundberg)

08:30 **Symposium 1**. Tree biotechnology in the postgenomic era

12:00 IUFRO 2005 Group Photo

12:15 Lunch and poster viewing

13:30 **Symposium 2**. Tree interactions with pests, pathogens and symbionts

19:00 Opening Gala Reception, hosted by the Mayor of the City of Tshwane

Tuesday - 8 November 2005

08:30 **Symposium 3**. Breakthrough and high-throughput technologies for functional and structural genomics in trees

12:00 Lunch and poster viewing

13:40 **Symposium 4**. Forest biotechnology adoption and the impact of the economic, scientific and societal value chains

19:00 African palette cuisine at the Sunset Boma (Overlooking the Cradle of Humankind), Blue Hills, Midrand,

Wednesday - 9 November 2005

07:00 *Eucalyptus* Genome Breakfast (Sanlam Auditorium Dining Hall)

07:30 Business Meeting of the International *Eucalyptus* Genome Consortium (SRC Hall)

08:30 **Symposium 5**. Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

12:00 Lunch and poster viewing

14:00 Visit De Wildt Cheetah Sanctuary (optional)

19:30 Traditional Afrikaner dinner and entertainment at Voortrekker Monument

Thursday - 10 November 2005

08:30 **Symposium 6**. Molecular biology and biotechnology of tree development

12:00 **Business** Meeting of IUFRO Working Party 2.04.06 (Molecular biology of forest trees)

12:30 Lunch and poster viewing

13:30 **Symposium 6.** (continue). Molecular biology and biotechnology of tree development

14:50 **Symposium 7**. (Parallel Session). From somatic embryos to genetic engineering

14:50 **Symposium 8**. Part I. (Parallel Session) Biotechnology and metabolic engineering of wood formation in trees

19:00 Gala Farewell Dinner at Pretoria Country Club

Friday - 11 November 2005

08:30 **Symposium 8**. Part II. Biotechnology and metabolic engineering of wood formation in trees

11:50 Lunch (All posters have to be removed by the end of lunch)

13:20 **Symposium 9**. Abiotic stress: Interaction of trees with the environment

16:50 Meeting ends

DETAILED SCIENTIFIC PROGRAMME

Sunday – 6 November 2005

13:30 Buses start departing from Conference Accommodation to the Conference Venue (Sanlam Auditorium) – three buses will depart from each hotel at the times indicated below

Bus No	1	2	3
Sheraton Hotel	13:30	15:15	17:00
Court Classique	13:45	15:30	17:15
Courtyard Arcadia	14:00	15:45	17:30
Holiday Inn Hatfield	14:15	16:00	17:45
Hatfield Manor Hotel	14:30	16:15	18:00
Hatfield Lodge	14:45	16:30	18:15

14:00 **Conference Secretariat opens**

Conference registration from 14:00 to 18:00

Poster boards available – all posters must be on display by 18:00

18:00 **Official welcoming on behalf of the University of Pretoria**

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18:30 Overview of Conference Schedule and General Information (Zander Myburg and Brenda Wingfield)

19:00 **Cheese & Wine Cocktail Welcome Reception at Sanlam Auditorium**

21:30 Buses depart for Conference Accommodation

Monday - 7 November 2005

07:30 Buses depart for Conference Venue (Sanlam Auditorium)

08:15 **Official welcoming on behalf of the IUFRO Working Party (Björn Sundberg)**

Symposium 1. Tree biotechnology in the postgenomic era

Chairs: Jerry Tuskan, Dario Grattapaglia (Venue, Sanlam Auditorium)

08:30	Amy Brunner	**Plenary presentation:** FROM GENOME SEQUENCE TO TRANSCRIPTOME: STUDIES USING A WHOLE-GENOME POPLAR MICROARRAY (S1.1)
09:00	Gerald Tuskan	**Plenary presentation:** THE POPULUS GENOME: GENOME-WIDE DUPLICATION, SUBFUNCTIONALIZATION AND PERENNIAL WOODY HABIT (S1.2)
09:30		**Tea and Coffee Break**
10:00	Simon Southerton	IDENTIFICATION OF GENES CONTROLLING WOOD FIBRE PROPERTIES IN *EUCALYPTUS NITENS* (S1.3)
10:20	Carlos Labate	PROTEOMIC ANALYSIS OF WOOD FORMATION AT DIFFERENT STAGES OF DEVELOPMENT OF *EUCALYPTUS GRANDIS* (S1.4)

10:40	Udaya Kalluri	FUNCTIONAL GENOMIC STUDIES OF AUXIN SIGNALING AND RESPONSE GENES IN *POPULUS* (S1.5)
11:00	Chung-Jui Tsai	A FUNCTIONAL GENOMICS INVESTIGATION OF CARBON PARTITIONING AMONG SECONDARY METABOLITE POOLS IN *POPULUS* (S1.6)
11:20	Jan Karlsson	UPSC-BASE – TREE TRANSCRIPTOMICS ONLINE (S1.7)
11:40	Georgios Pappas	SAMPLE SEQUENCING OF 3 MEGABASES OF SHOTGUN DNA OF *EUCALYPTUS GRANDIS*: GENOME STRUCTURE, REPETITIVE ELEMENTS AND GENES (S1.8)

12:00 IUFRO 2005 Group Picture (all attend please)

12:15 Lunch and poster viewing (odd numbers manned)

Symposium 2. Tree interactions with pests, pathogens and symbionts

Chairs: Jan Stenlid, Mike Wingfield (Venue, Sanlam Auditorium)

13:30	Louis Bernier	**Plenary presentation:** GENOME EXPLORATION IN THE DUTCH ELM DISEASE FUNGUS, *OPHIOSTOMA NOVO-ULMI* (S2.1)
14:00	Jan Stenlid	**Plenary presentation**: RECENT STUDIES IN THE CONIFER - *HETEROBASIDION* PATHOSYSTEM (S2.2)
14:30	Guillermo Salvatierra	TRANSCRIPTIONAL PROFILE OF THE METABOLIC RESPONSE OF *EUCALYPTUS GRANDIS* TO THE FUNGAL PATHOGEN *PUCCINIA PSIDII* (S2.3)
14:50	Carl Gunnar Fossdal	DEFENSE REACTIONS IN NORWAY SPRUCE TOWARD THE PATHOGENIC ROOT-ROT CAUSING FUNGUS *HETEROBASIDION ANNOSUM* (S2.4)
15:10		**Tea and Coffee Break**
15:40	Elisabeth Magel	REGULATION OF CARBON TRANSFER IN THE MISTLETOE-HOST (POPLAR) INTERFACE (S2.5)
16:00	Bongani Maseko	SCREENING AND SELECTION OF HALF-SIB FAMILIES OF *EUCALYPTUS SMITHII* FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI* AND *P. NICOTIANAE* (S2.6)
16:20	Armand Séguin	INTERACTING GENOMES IN TREE-PEST RESPONSE (S2.7)
16:40	V. Legué	STUDIES OF SIGNALLING PATHWAYS IN RESPONSE TO HYPAPHORINE AND TO HYPAPHORINE/IAA COMPETITIVE INTERACTION IN *EUCALYPTUS* AND POPLAR ROOTS (S2.8)

17:15 Buses depart for Conference Accommodation

18:15 Buses depart for Evening Social Programme

19:00 Opening Gala Reception, hosted by the Mayor of the Metropolitan City of Tshwane

22:00 Buses start departing for Conference Accommodation

Tuesday - 8 November 2005

07:30 Buses depart for Conference Venue (Sanlam Auditorium)

Symposium 3. Breakthrough and high-throughput technologies for functional and structural genomics in trees

Chairs: Magnus Hertzberg, Joerg Bohlmann (Venue, Sanlam Auditorium)

08:30	Thomas Moritz	**Plenary presentation:** METABOLITE PROFILING OF *POPULUS*: INSTRUMENTATION, CHEMOMETRICS AND DATABASES FOR INTEGRATION IN TREE BIOTECHNOLOGY (S3.1)
09:00	John MacKay	**Plenary presentation:** A FUNCTIONAL GENOMICS APPROACH TO IDENTIFYING

		POTENTIAL REGULATORS OF TREE GROWTH, DEVELOPMENT AND DEFENSE RESPONSES (S3.2)
09:30		**Tea and Coffee Break**
10:00	Joerg Bohlmann	THE NEED FOR HIGH-THROUGHPUT BIOCHEMICAL GENOMICS: TERPENOID SYNTHASES AND P450 ENZYMES IN CONIFER DEFENSE (S3.3)
10:20	Armin Wagner	FUNCTIONAL GENE TESTING IN *PINUS RADIATA* CALLUS CULTURES (S3.4)
10:40	Timothy Tschaplinski	COMBINING METABOLOMICS AND QTL ANALYSIS FOR IDENTIFYING MQTL AND GENE DISCOVERY IN POPLAR (S3.5)
11:00	Fredrik Sterky	CHARACTERIZATION OF REGULATORY GENES IN THE SECONDARY MERISTEM OF *POPULUS* BY IN SITU PROTEIN LOCALIZATION (3.6)
11:20	Dustin Lippert	THE INTRICACIES AND INCENTIVES OF HIGH-THROUGHPUT PROTEOMICS IN ITS APPLICATION TO TREE BIOLOGY (S3.7)
11:40	Sergio Brommonschenkel	A BAC LIBRARY OF *EUCALYPTUS GRANDIS*: CHARACTERIZATION, FINGERPRINTING, BAC-END SEQUENCING AND SHOTGUN ASSEMBLY OF LIGNIFICATION GENES (S3.8)

12:00 **Lunch and poster viewing (even numbers manned)**

Symposium 4. Forest biotechnology adoption and the impact of the economic, scientific and societal value chains

Chairs: Maude Hinchee and Susan McCord (Venue, Sanlam Auditorium)

	The Economic Value Chain	
13:40	Peter Farnum	(Weyerhaeuser Company) HOW BIOTECHNOLOGY WILL IMPACT THE FOREST PRODUCTS INDUSTRY (S4.1)
14:05	Maud Hinchee	(ArborGen, LLC) THE BENEFIT OF THE APPLICATIONS OF FOREST BIOTECHNOLOGY (S4.2)
	The Scientific Value Chain	
14:30	Ove Nilsson	(Umeå Plant Science Centre) BASIC KNOWLEDGE EVOLUTION THROUGH TREE BIOTECHNOLOGY RESEARCH (S4.3)
14:55	Timothy Strabala	(Scion Research) AN ASSOCIATION GENETICS AND FUNCTIONAL GENE TESTING PIPELINE FOR TREE IMPROVEMENT (S4.4)
15:20		**Tea and Coffee Break**
	The Societal Value Chain	
15:40	Marie Connett	(CAMBIA) UNBLOCKING THE OBSTACLES TO OPEN USE (S4.5)
16:05	Steven Burke	(North Carolina Biotechnology Center) ADOPTION OF FOREST BIOTECHNOLOGY WORLDWIDE: PROCESS, IMPLICATIONS, AND SOCIETAL ISSUES (S4.6)
16:30	Speaker Panel	QUESTION AND ANSWER SESSION: KEY QUESTIONS ON FOREST BIOTECHNOLOGY ADOPTION (PANEL DISCUSSION LED BY SUSAN MCCORD, INSTITUTE OF FOREST BIOTECHNOLOGY)

17:15 Buses depart for Conference Accommodation

Optional Evening Programme

18:15 Buses depart for Evening Social Programme

19:00 African palette cuisine at the Sunset Boma (Overlooking the Cradle of Humankind), Blue Hills, Midrand,

22:00 Buses start departing for Conference Accommodation

Wednesday - 9 November 2005

06:30 Early buses depart for International *Eucalyptus* Genome Consortium meeting

07:00 *Eucalyptus* Genome Breakfast (Sanlam Auditorium Reception Area)

07:30 **Business Meeting of the International *Eucalyptus* Genome Consortium (SRC Council Chamber)**

07:30 Regular buses depart for Conference Venue (Sanlam Auditorium)

Symposium 5. Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

Chairs: Christophe Plomion, Matias Kirst (Venue, Sanlam Auditorium)

08:30	Michele Morgante	**Plenary presentation:** SEQUENCE DIVERSITY IN PLANTS: IS THERE FUNCTIONAL VARIATION BEYOND SNPS? (S5.1)
09:00	Jerry Tuskan (for Stephen DiFazio)	**Plenary presentation:** TOOLS AND STRATEGIES FOR IDENTIFYING CANDIDATE GENES FOR COMPLEX TRAITS IN *POPULUS* (S5.2)
09:30		**Tea and Coffee Break**
10:00	Brad Potts	MOLECULAR INSIGHTS INTO THE GENE POOL OF *EUCALYPTUS GLOBULUS* (S5.3)
10:20	Dudley Huber	ASSOCIATION GENETICS IN PINE – THE ADEPT2 PROJECT (S5.4)
10:40	Bala Thumma	ASSOCIATION STUDIES IN *EUCALYPTUS* SPP (S5.5)
11:00	Dana Nelson	TOWARDS A COMPLETE LOBLOLLY PINE GENETIC MAP FOR APPLICATION IN MARKER-DIRECTED POPULATION IMPROVEMENT (MDPI) (S5.6)
11:20	Betty Pelgas	COMPARATIVE MAPPING WITHIN THE GENUS *PICEA* AND ANALYSIS OF SYNTENY WITH OTHER PINACEAE (S5.7)
11:40	Steve Hanley	GENETIC ANALYSIS OF COMPLEX TRAITS IN WILLOW (S5.8)

12:00 Lunch and poster viewing (odd numbers manned)

13:30 Buses depart for Conference Accommodation (Attendees that do not go on the optional afternoon trip)

Optional Afternoon Programme

12:30 Buses depart for De Wildt Cheetah Sanctuary

13:30 Visit De Wildt Cheetah Sanctuary

16:30 Buses depart for Conference Accommodation

Optional Evening Programme

18:30 Buses depart for Evening Social Programme

19:00 Traditional Afrikaner dinner and entertainment at Voortrekker Monument

21:30 Buses start departing for Conference Accommodation

Thursday - 10 November 2005

07:30 Buses depart for Conference Venue (Sanlam Auditorium)

Symposium 6. Molecular biology and biotechnology of tree development

Chairs: Antje Rohde, Amy Brunner and Rishi Bhalerao (Venue, Sanlam Auditorium)

08:30	Andrew Groover	**Plenary presentation:** GENETIC MECHANISMS REGULATING THE VASCULAR CAMBIUM AND SECONDARY GROWTH (S6.1)
09:00	Rishikesh Bhalerao	**Plenary presentation:** ANALYSIS OF REGULATION OF CAMBIAL MERISTEM ACTIVITY USING A GENOMICS APPROACH (S6.2)
09:30		**Tea and Coffee Break**
10:00	Antje Rohde	THE DORMANCY TRANSCRIPTOME IN APICAL BUDS OF POPLAR (S6.3)
10:20	Daniel Eriksson	DIURNAL RHYTHMS OF TRANSCRIPTOME AND METABOLOME DURING PHOTOPERIODIC REGULATION OF SHOOT GROWTH CESSATION IN *POPULUS* (S6.4)
10:40	Henrik Böhlenius	REGULATION OF FLOWERING TIME IN TREES (S6.5)
11:00	Jeanette Nilsson	TRANSCRIPTIONAL CONTROL OF CELL IDENTITY AND DIFFERENTIATION IN THE VASCULAR CAMBIUM IN RELATION TO SEASONAL CHANGES AND GROWTH ACTIVITY (S6.6)
11:20	Taku Demura	MASTER REGULATORS OF PROTOXYLEM AND METAXYLEM VESSEL FORMATION (S6.7)
11:40	Gerd Bossinger	UNDERSTANDING CAMBIAL DEVELOPMENT AND WOOD FORMATION IS A PATCHY BUSINESS (S6.8)

12:00 Business Meeting of IUFRO Working Party 2.04.06 (Molecular biology of forest trees)

Presentation of bid committees for next meeting

Election of next working party coordinator and deputies

12:30 Lunch and poster viewing (even numbers manned)

Symposium 6. (continued) Molecular biology and biotechnology of tree development

13:30	Charleen Moreau	CHARACTERIZATION OF DEVELOPMENTAL PROGRAMMED CELL DEATH IN XYLEM FIBERS OF POPLAR (*POPULUS TREMULA* X *TREMULOIDES*): FROM ANATOMICAL TO MOLECULAR INSIGHTS INTO THE DEATH PROCESS OF WOOD (S6.9)
13:50	Ulrika Egertsdotter	GENOMICS OF CONIFER EMBRYO DEVELOPMENT (S6.10)
14:20		**Tea and Coffee Break**

Symposium 7. (Parallel Session) From somatic embryos to genetic engineering

Chairs: Ulrika Egertsdotter and Rick Meilan (Venue: SRC Council Chamber)

14:50	Kurt Zoglauer	**Plenary presentation:** AUXIN SIGNALING DURING PATTERN FORMATION OF SOMATIC EMBRYOS OF *LARIX DECIDUA* (S7.1)
15:20	Carmen Diaz-Sala	IDENTIFICATION OF GENES RELATED TO ADVENTITIOUS ROOTING CAPACITY IN PINE AND CHESTNUT (S7.2)
15:40	Tuija Aronen	FIELD PERFORMANCE OF SCOTS PINE CUTTINGS OF FASCICULAR SHOOT ORIGIN ROOTED BY *AGROBACTERIUM* (S7.3)
16:00	Etsuko Matsunaga	EFFICIENT TRANSFORMATION METHOD OF *EUCALYPTUS GLOBULUS* AND *EUCALYPTUS CAMALDULENSIS* (S7.4)
16:20	Célia Miguel	PPRAB1, A RAB-RELATED SMALL GTP-BINDING PROTEIN IS PRESENT IN *PINUS PINASTER* AND IS EXPRESSED PREDOMINANTLY IN EARLY EMBRYOGENESIS AND SEEDLINGS (S7.5)

16:40	Mariana Vetrici	THE DOUGLAS-FIR *LEAFY COTYLEDON1* (*PMLEC1*) GENE IS A TRANSCRIPTION FACTOR EXPRESSED DURING EARLY SOMATIC AND ZYGOTIC EMBRYOGENESIS AND IS INDUCIBLE BY STRESS AND HORMONES (S7.6)

Symposium 8. Part I. (Parallel Session) Biotechnology and metabolic engineering of wood formation in trees

Chairs: Wout Boerjan, Björn Sundberg (Venue, Sanlam Auditorium)

14:50	Lise Jouanin	**Plenary presentation**: USE OF AN *ARABIDOPSIS* CAD MUTANT TO DETERMINE THE FUNCTION OF CADS OF *ARABIDOPSIS* AND TREE ORIGIN (S8.1)
15:20	Yasushi Sato	CHARACTERISATION OF A TEMPERATURE-SENSITIVE MUTANT OF *ARABIDOPSIS*, LIG, WHICH EXHIBITS ABERRANT LIGNIN DEPOSITION AND GROWTH DEFECTS (S8.2)
15:40	Deborah Goffner	STRATEGIES FOR IDENTIFYING DETERMINANTS OF SECONDARY CELL WALL FORMATION USING MODEL SYSTEMS (S8.3)
16:00	Ellinor Edvarsson	USE OF POPLAR AND *ARABIDOPSIS* MODEL SYSTEMS TO IDENTIFY GENES INVOLVED IN WOOD FORMATION (S8.4)
16:20	Jacqueline Grima-Pettenatti	NEW MYB TRANSCRIPTION FACTORS FROM *EUCALYPTUS* XYLEM REGULATE SECONDARY CELL WALL FORMATION AND LIGNIN DEPOSITION (S8.5)
16:40	Toshiaki Umezawa	METABOLIC PROFILING OF THE CINNAMATE/MONOLIGNOL PATHWAY BY THE USE OF STABLE-ISOTOPE-DILUTION METHOD (S8.6)

17:15 Buses depart for Conference Accommodation

18:15 Buses depart for Evening Social Programme

19:00 Gala Farewell Dinner at Pretoria Country Club

22:00 Buses start departing for Conference Accommodation

Friday - 11 November 2005

07:30 Buses depart for Conference Venue (Sanlam Auditorium)

Symposium 8. Part II. Biotechnology and metabolic engineering of wood formation in trees

Chairs: Wout Boerjan, Björn Sundberg (Venue, Sanlam Auditorium)

08:30	Tuula Teeri	**Plenary presentation:** CARBOHYDRATE ACTIVE ENZYMES IN THE HYBRID ASPEN XYLEM (S8.7)
09:00	Joshi Chandrashekhar	THE WAYS AND MEANS OF BOOSTING CELLULOSE PRODUCTION IN TRANSGENIC TREES (S8.8)
09:20	Björn Sundberg	ENGINEERING OF WOOD STRUCTURES (S8.9)
09:40		**Tea and Coffee Break**
10:10	Wout Boerjan	DOWN-REGULATION OF CINNAMOYL-COA-REDUCTASE IN POPLAR: FROM THE LAB TO THE FIELD (S8.10)
10:30	Johan Wadenbäck	NATURAL VARIATION IN LIGNIN AND THE INFLUENCE OF TRANSGENIC EXPRESSION IN LIGNIN BIOSYNTHESIS IN YOUNG WOOD OF NORWAY SPRUCE (S8.11)
10:50	Heather Coleman	THE IMPACT OF RNAI-MEDIATED SUPPRESSION OF P-COUMARYLSHIKIMATE 3'-HYDROXYLASE EXPRESSION ON LIGNIN CONTENT AND STRUCTURE IN POPLAR (S8.12)
11:10	Ewa Mellerowicz	PECTIN METHYLESTERIFICATION AFFECTS MANY ASPECTS OF WOOD CELL DEVELOPMENT (S8.13)
11:30	Oded Shoseyov	GROWTH ENHANCEMENT AND WOOD FIBER IMPROVEMENT BY CELL-WALL MODULATING PROTEINS (S8.14)

11:50 Lunch

Symposium 9. Abiotic stress: Interaction of trees with the environment

Chairs: Arie Altman, Andrea Polle (Venue, Sanlam Auditorium)

13:20	Jeffrey Dean	**Plenary presentation:** USING FUNCTIONAL GENOMICS TO DISSECT DROUGHT RESPONSES IN PINE (S9.1)
13:50	Christophe Plomion	**Plenary presentation**: INSIGHT INTO THE GENETIC CONTROL OF MOLECULAR PLASTICITY: A CASE OF STUDY IN MARITIME PINE (S9.2)
14:20	Matthias Arend	REGULATION OF WOOD GROWTH BY ABA IN DROUGHT STRESSED POPLAR (S9.3)
14:40	Basia Judith Vinocur	THE POSSIBLE ROLE OF SP1 PROTEIN IN UNRAVELING SALT STRESS TOLERANCE IN *POPULUS EUPHRATICA* OLIV., A NEW HYDRO-HALOPHYTE MODEL TREE SPECIES (S9.4)
15:00	**Tea and Coffee Break (All posters have to be removed by the end of break)**	
15:30	Andrea Polle	FROM GENES TO FUNCTION: HOW DOES *POPULUS EUPHRATICA* COMPENSATE SALT-INDUCED OSMOTIC STRESS? (S9.5)
15:50	Jason Holliday	FUNCTIONAL AND POPULATION GENOMICS OF COLD ACCLIMATION IN *PICEA SITCHENSIS* (9.6)
16:10	Christiane Marque	CBF TRANSCRIPTION FACTORS IN *EUCALYPTUS* COLD TOLERANCE (S9.7)
16:30	Carsten Kulheim	PHYSIOLOGICAL AND MOLECULAR CHANGES IN RNAI MUTANT ASPEN TREES, LACKING THE PSBS PROTEIN (S9.8)

16:50 Meeting ends

17:00 Buses depart for Conference Accommodation (Earlier buses will be arranged for people that need to leave earlier)

Buses depart for Airport (Scheduled on an individual basis)

SOCIAL PROGRAMME OVERVIEW

Date	Time	Venue	Details	Dress Code	Remarks	Cost
Sunday 06/11/2005	19:00	Sanlam Auditorium, University of Pretoria	Welcome Cheese & Wine Cocktail Reception	Smart Casual	All delegates and registered accompanying persons are welcome	Transport R60 per person (if not paid in registration fee)
Monday 07/11/2005	19:00	Sammy Marks Convention Centre	City of Tshwane Metropolitan Municipality Mayoral Opening Banquet	Formal	All delegates and registered accompanying persons are welcome	Transport R60 per person (if not paid in registration fee)
Tuesday 08/11/2005	19:00	Sunset Boma, Blue Hills – Midrand	An African palette, cuisine extravaganza.	Smart/casual	Only delegates and accompanying persons who registered and paid for this optional event	R 400.00 per person including transport R 340 own transport
Wednesday 09/11/2005	12:00	De Wildt Cheetah Sanctuary	See the very rare King Cheetah, wild dogs, brown hyena, blue duiker, suni antelope and species of vultures and owls	Casual Hats, comfortable shoes, water bottles recommended	Only delegates and accompanying persons who registered and paid for this optional event	R500 per person including transport
	19:00	Voortrekker Monument	Traditional Afrikaner meal and traditional folk dancing "volkspele" and guided tour of the Monument. One free glass of wine per delegate with the dinner. Cash bar	Smart casual	Only delegates and accompanying persons who registered and paid for this optional event	R275 per person including transport R 215 own transport
Thursday 10/11/2005	19:00	Pretoria Country Club	Gala dinner – traditional "potjie kos". Entertainment by the Soccajasco Kids – African musicians	Smart/casual	All delegates and registered accompanying persons are welcome	Transport R60 per person (if not paid in registration fee)

ABSTRACTS

S1. Tree biotechnology in the postgenomic era

S2. Tree interactions with pests, pathogens and symbionts

S3. Breakthrough and high-throughput technologies for functional and structural genomics in trees

S4. Forest biotechnology adoption and the impact of the economic, scientific and societal value chains

S5. Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

S6. Molecular biology and biotechnology of tree development

S7. From somatic embryos to genetic engineering

S8. Biotechnology and metabolic engineering of wood formation in trees

S9. Abiotic stress: Interaction of trees with the environment

S1. Tree biotechnology in the postgenomic era

S1.1

FROM GENOME SEQUENCE TO TRANSCRIPTOME: STUDIES USING A WHOLE-GENOME POPLAR MICROARRAY

Palitha Dharmawardhana[1], Stephen P. DiFazio[2], Lee Gunter[2], Amy M. Brunner[1]

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The completion of the *Populus trichocarpa* genome sequence and initial annotation has enabled development of powerful genomic tools for understanding tree biology and facilitating tree improvement. Our goal was to develop a whole-genome poplar microarray that performed well with all *Populus* species and hybrids. 60mer oligonucleotides were designed for all *P. trichocarpa* gene models as well as divergent ESTs from aspen species and hybrids. The first generation microarray represents 65,966 individual poplar sequences including nuclear, organelle and microRNA precursor genes. Initial transcriptome studies compare a variety of *P. trichocarpa* tissue types and developmental stages. For example, comparison of differentiating xylem and phloem during late summer revealed nearly 4,000 differentially expressed genes. Using both RNA and genomic DNA hybridization, we are also comparing array performance with *P. trichocarpa* versus a *P. tremula x P. alba* genotype commonly used for transformation. Additional results to be presented include total number of genes detected as expressed by functional classification, and expression patterns of select gene families in a phylogenetic context that includes examples of recently duplicated genes exhibiting differential expression.

S1.2

THE POPULUS GENOME: GENOME-WIDE DUPLICATION, SUBFUNCTIONALIZATION AND PERENNIAL WOODY HABIT

Gerald A. Tuskan[1], S. P. DiFazio[1], U. Hellsten[2], J. Chapman[2], I. Dubchak[2], S. Jawdy[1], U. Kalluri[1], S. Jansson[3], D. Rokhsar[2]

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The *Populus* Genome has experienced a recent genome-wide duplication and it shares a more ancient genome-wide duplication with *Arabidopsis*. Both the ancient and recent events are detectable at the nucleotide level, at the gene level, and at the whole-chromosome level. Roughly, 92% of genome is covered by segments from the recent duplication and 59% of genome is covered by segments from the ancient duplication. Approximately, 39% of genes occur as two recent copies and 16% occur as ancient pairs. These events, and continued expansion of some gene families, have led to noticeable difference in gene family and gene domains between *Populus* and *Arabidopsis*. Comparisons based on PFAM and KOG domains and tribe analysis suggests that *Populus* has between 1.63 and 1.88 genes per domain or tribe relative to *Arabidopsis*. Several of these differences reflect the alternate life histories of the two organisms – *Populus* a dioecious perennial and *Arabidopsis* a hermaphroditic annual. That is, *Populus* has an overrepresentation of domains involved in plant-microbe interactions, including pathogenic and symbiotic genes. *Arabidopsis* has an overrepresentation of self-compatibility recognition and pollen coat genes. *Populus* also has an abundance of gene involved in programmed cell death, possibly associated with intra-annual leaf abscission, fine root turnover and dormancy. Statistical analysis of electronic Northern data also suggests that the duplicated genes in *Populus* are differentially expressed in alternate tissues.

S1.3

IDENTIFICATION OF GENES CONTROLLING WOOD FIBRE PROPERTIES IN *EUCALYPTUS NITENS*

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We analyzed patterns of gene expression and variation in a number of wood properties in wood forming in the upper and lower sides of branches and in vertical stems of *Eucalyptus nitens*. Cellulose and lignin content, cellulose microfibril orientation, basic density, cellulose crystallinity and crystallite width were measured in wood samples taken from branches and vertical stems. RNA isolated from developing xylem from branches and vertical stems was used to screen microarrays containing approximately 4500 cDNA clones from a *Eucalyptus grandis* xylem cDNA library. Cellulose content in wood forming on the upper side of branches was significantly higher, while cellulose content was significantly reduced in wood forming on the underside of branches, when compared to vertical stems. Klason lignin levels revealed an opposite trend to cellulose content. Cellulose microfibril angle was significantly lower in wood from the upper side of the branch and higher in wood forming on the underside of branches, compared to vertical stems. A cellulose synthase gene was identified that was more strongly expressed in upper branch xylem. The most strongly up regulated genes in upper branches included two arabinogalactan proteins, while one of these genes was also strongly down regulated in xylem from the lower side of branches. A large number of genes were observed to be differentially expressed in vertical stems and branches.

300 unrelated Victrie population

alcian blue – insoluble carb.

violet cristal – lignin

TUBULINS AND MICROTUBULES MAY INFLUENCE CELLULOSE MF ORIENTATION

S1.4

PROTEOMIC ANALYSIS OF WOOD FORMATION AT DIFFERENT STAGES OF DEVELOPMENT OF *EUCALYPTUS GRANDIS*

Carlos A. Labate[1], Alexander de Andrade[1], Paola F. Celedon[1], Karem G. Xavier[1], Shinitiro Oda[2]

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Wood is formed in trees as a consequence of secondary growth of the vascular cambium located between the xylem and phloem. Despite of its economical importance for the pulp and paper industry, timber and energy production, very little is known about the genetic control of wood formation. We have started a functional genomics project for parallel identification of genes (using the SAGE technology) and proteins involved in wood formation of *Eucalyptus grandis* at three stages of development: 0-6 months, 2.7 years and 5.5 years. Tissues from cambial zone at each stage of development were isolated for total RNA and proteins extraction. Two-dimensional gels electrophoresis were reproducibly prepared with denaturing isoelectric focusing in the first dimension (pH ranges 3.0-10 and 4.0-7.0) and SDS-PAGE in the second dimension. Gels were stained with Coomassie Brilliant Blue (R-250) and scanned for image analysis. Individual protein spots were selected and excised from the gel and digested with site-specific protease trypsin, resulting in a set of tryptic peptides. The peptides were separated in a Symetry C18 5µm (0.32 x 150mm) column and amino acid sequence obtained using a nanoelectrospray tandem MS (ESI-MS/MS) Micromass Q-TOF Ultima. The peptides sequences were used to search the public databases (NCBI and SwissProt). Different classes of proteins were identified at each stage of development, with the majority of proteins involved in primary and secondary metabolism, cell wall formation, disease and stress response. The results will be presented comparing the gene and protein expression.

Separation of proteins in 2-D Gels and de novo sequencing by LC-MSMS

CLONES SEQUENCED 1.800

S1.5

FUNCTIONAL GENOMIC STUDIES OF AUXIN SIGNALING AND RESPONSE GENES IN *POPULUS*

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Aux/IAA (Auxin/Indole 3- Acetic Acid) and *ARF* (Auxin Response Factor) genes are key regulators of auxin responses in plants. We explored the extent of conservation and divergence between *Populus* and *Arabidopsis* with respect to these two large gene families. There are 40 *ARF* genes (*PoptrARF*) predicted in the *Populus* genome compared to 23 *ARF* genes (*AtARF*) predicted in the *Arabidopsis* genome. The domain architectures of the predicted PoptrARF proteins are largely conserved with the orthologous AtARFs, however, phylogenetic analysis shows divergence in the expansion of certain ARF subgroups like ARFs 6, 16, 3, 9 and 2. The ratio of activator ARFs (possessing Q-rich middle regions) in *Arabidopsis* and *Populus* is 5:13 whereas the ratio of other ARFs is 18:27 indicating an enrichment of activator ARFs during *Populus* evolution. The *Populus* genome has 35 *Aux/IAA* genes (*PoptrIAA*), while the *Arabidopsis* genome contains 29 *Aux/IAA* genes (*AtIAA*). Four groups of PoptrIAAs (PoptrIAA3, PoptrIAA29, PoptrIAA16 and PoptrIAA27) have expanded to contain 3 to 4 members each. Gene-specific expression studies showed that the *PoptrIAA3* co-orthologs display differential expression in major plant organs. This preliminary observation towards functional divergence following selective retention of duplicated genes was extended to the global transcriptome scale by the use of whole genome oligoarrays. In addition, auxin responses also were studied at the molecular level in time-dose series experiments. Last but not the least, several *Aux/IAA* RNAi transgenics were generated to address our goal to elucidate functional roles of these genes in the context of *Populus* biology. Results from these functional genomic studies will be discussed.

S1.6

A FUNCTIONAL GENOMICS INVESTIGATION OF CARBON PARTITIONING AMONG SECONDARY METABOLITE POOLS IN *POPULUS*

Chung-Jui Tsai[1], Scott Harding[1], Yinan Yuan[1], Han-Wei Lin[1], Changyu Hu[1], Jingwei Yin[1], Robert Sykes[2], Mark Davis[2]

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Populus species accumulate non-nitrogenous secondary metabolites, predominantly phenylpropanoid-derived phenolic glycosides (PGs) and condensed tannins (CTs) in their leaves, roots and bark. PGs and CTs, together with vascular lignin, comprise the vast majority of secondary carbon in these tissues. In leaves, PGs and CTs exhibit wide variation in composition and quantity, and can comprise more than 35% of dry weight. How carbon is distributed among these sinks impacts tree fitness, growth, and utilization of tree species for long-term sequestration of atmospheric carbon dioxide. High-throughput technologies, including microarray, GC-MS, NIR and pyMBMS profiling, are being used to analyze phenolic regulation in cell suspension cultures and hybrid cottonwood lines exhibiting a wide range of foliar PG and CT concentrations. Wounding and nitrogen stress were used as tools to perturb phenylpropanoid carbon allocation and partitioning. Responses included no change, and inducible CT or PG accumulation, in some cases significant enough to affect biomass growth. Synthesis of salicylate-derived PGs appears to depend on pathways both up- and down-stream of PAL, and the use of whole-genome microarrays is expected to help elucidate the participation of these pathways in PG biosynthesis. Although the flavonoid biosynthetic pathway has been well characterized in model species, comparative analysis of *Populus* and *Arabidopsis* genomes revealed large disparities in the structural gene network leading to CT biosynthesis. Consistent with greater abundance and complexity of *Populus* CTs, RT-PCR analysis revealed differential regulation of flavonoid pathway genes in CT-rich root and young shoot tissues of *Populus*. Other data point to qualitative differences between root and shoot CTs that could be related to the observed differences in gene expression pattern. Continued functional genomics analysis of PG and CT metabolism should improve our ability to exploit the wide natural variation of these pools for enhanced productivity and global carbon management through biotechnology.

S1.7

UPSC-BASE – TREE TRANSCRIPTOMICS ONLINE

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The increasing accessibility and use of microarrays in transcriptomics has accentuated the need for purpose-designed storage and analysis tools. Here, we present UPSC-BASE, a database for analysis and storage of *Populus* DNA microarray data. A microarray analysis pipeline has also been established to allow consistent and efficient analysis (from small to large scale) of samples in various experimental designs. A range of optimised experimental protocols is provided for each step in the generation of the data. Within UPSC-BASE, researchers can perform standard and advanced microarray analysis procedures in a user-friendly environment. Background corrections, normalisations, quality control tools, visualisations, hypothesis tests and export tools are provided without requirements for expert-level knowledge. Although the database has been developed primarily for handling *Populus* DNA microarrays, most of the tools are generic and can be used with minor modifications in all kinds of BASE installations, regardless of the organism and microarray system concerned. UPSC-BASE is also a repository of *Populus* microarray information, providing data from over 25 experiments on a total of more than 800 microarray slides in the public domain of the database. We believe that the description of the publicly accessible database will increase the attraction of *Populus* as a model system for molecular biology, genetics and genomics.

S1.8

SAMPLE SEQUENCING OF 3 MEGABASES OF SHOTGUN DNA OF *EUCALYPTUS GRANDIS*: GENOME STRUCTURE, REPETITIVE ELEMENTS AND GENES

Rodrigo T. Lourenço[1,2], Dario Grattapaglia[2,3], Georgios J. Pappas Jr[3], Gonçalo A. Pereira[1]

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To obtain a general overview of the structure and composition of the *Eucalyptus grandis* genome, we sample sequenced 10,000 randomly sheared genomic DNA clones. After filtering for quality and length (phred value >=20; length >=200 bases) 7,395 reads representing roughly 3,0 Mbp of sequence were analyzed for their nucleotide content, repetitive elements and gene content. The program RepeatMasker was used to analyze the %GC content and repetitive patterns and elements. Results indicate that on average the *Eucalyptus* genome has a GC content of 40.15% with 38.95% in introns and 45.51% in exons. From the total bases sequenced approximately 1.4% were located in transposons, distributed in 310 interspersed repetitive genetic elements, among which 299 classified as retroelements, mainly LTRs with 244 copia-like elements and 52 gypsy-like. We also identified 1,636 low complexity sequences and 987 microsatellites including both perfect and interrupted, with 18.2% mono, 39.3% di, 16.8% tri, 10.3% tetra, 11.3% penta and 4.0% hexanucleotide repeats with the (TC)n motif being the most abundant (26%) and an estimated frequency of one microsatellite every 3 kbp in the *Eucalyptus* genome. With a more stringent analysis using the software TROLL, 319 di, tri, tetra and pentanucleotide perfect repeats were found resulting in a frequency of one perfect microsatellite every 12 kbp in the *Eucalyptus* genome. Primer pairs could be designed for 156 markers out of which 93 of them were selected for fluorescent labelling and mapping after screening for amplification and polymorphism. In total we estimated that only 5.8% of the *Eucalyptus* genome is represented by repetitive elements, way lower than the 10% in *Arabidopsis* possibly due to the existence of elements not yet described and/or exclusive to *Eucalyptus*. To identify putative genes we used an alternative approach by comparing the genomic sequences with a *Eucalyptus* ESTs database using the GenESTate software. We attributed putative functions using a pipeline were the exons of each gene were put together and compared with protein domains data banks. This procedure avoids the misleading results obtained when comparing DNA sequences with sequences deposited in GenBank. The sequences were clustered using the CAP3 software, resulting in 766 contigs and 5,428 singlets, the former showing an average of 1,200 bp. These 766 contigs were compared with a relatively reduced set of available ESTs at the time (~5,000 *E. grandis* ESTs from mature leaf tissue and ~6,000 *E. urophylla* ESTs from xylem). From the 766 contigs we found 44 that showed high similarity to some ESTs. The coding portion of the sequences accounted for around 2% of the total sequences. It is important to highlight that by this approach it was possible to identify introns and exons, beside core promoter regions, which can't be identified in the ESTs. Other 166 possible genes were identified, 76 of them classified as housekeeping, among the genomic sequences by using blastx-nr in NCBI. We also identified putative genes responsible for 16 tRNAs using the tRNAscan-SE software. Besides providing a first glimpse at the general organization of the *Eucalyptus* genome, this database of sequences has provided a new randomly distributed source of microsatellites for genetic mapping.

S1.9p

THE STUDY OF THE GENETIC EXPRESSION PROFILE OF CAMBIAL TISSUE FROM ADULT *EUCALYPTUS GRANDIS* TREES USING SAGE TECHNOLOGY (SERIAL ANALYSIS OF GENE EXPRESSION)

Mayra C.C.G. de Carvalho[1], Danielle G. Gomes[1], Guillermo R. Salvatierra[1], Raphael T. Carneiro[1], David H. Moon[1], Shinitiro Oda[2], Carlos A. Labate[1]

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The great economic importance of wood has induced genome sequencing projects for *Populus* and *Eucalyptus* species. However, the accumulation of gene sequences represents only the first step in studies into wood formation. The comprehension of the events leading to secondary growth also requires information about positional and temporal gene expression. In this study we used SAGE to access the population of genes transcribed in cambial tissue from *E. grandis* trees. To make the library a bulk sample was produced containing the cambial tissue from 30 individual trees close to felling age (approximately 6 years old) from a commercial plantation. After the preliminary sequencing of 400 clones, a total of 13001 tags were recovered of which, 7507 tags with a frequency of 2 representing 1750 genes and 5494 singletons. The most expressed tags (410) were identified using NCBI databases; only 5 tags were not encountered in the databases. After a functional classification of the 410 genes the largest group was those without homology or hypothetical sequences (27%), categories associated with energy production and cellular maintenance (23%) and cellular regulation and development (18%). The categories involved in macromolecular metabolism and cellular structures had frequencies very close to one another (14% and 12%, respectively), with the transport category containing the fewest number of genes. This study is part of a larger project involving the production of two other SAGE libraries, from cambial tissue from other developmental stages (1-6 months, 1-3 years) *E. grandis* trees, and protein (proteomic) analysis from the three age groups. At present we are increasing the number of clones sequenced in an attempt to reduce the number of singletons and subsequently increase or confidence in the data generated. Another 200 clones will be sequenced (600 in total) generating another 6000 tags. This large project will permit, not only the identification of genes, proteins and factors that regulate secondary growth at the different growth phases of *E. grandis*, but also understand the molecular mechanisms that are involved in the determination of the structural characteristics of wood.

S1.10p

SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) OF THE CAMBIAL REGION OF JUVENILE *EUCALYPTUS GRANDIS* TREES

G. Gomes[1], Guillermo R. Salvatierra[1], Raphael T. Carneiro[1], Mayra C.C.G. de Carvalho[1], David H. Moon[1], Shinitiro Oda[2], Carlos A. Labate[1]

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Eucalyptus species have been intensively studied because of their importance in the production of cellulose and paper in Brazil. In 2004, 9.5 million MT of cellulose paste and 8.2 million MT of paper were produced, with 148455.7 hectares of reforested areas recorded in 2003. Due to the huge quantity of *Eucalyptus* wood produced, recent anatomical, morphological, cellular and molecular studies have turned their attention to the xylem as a collection of cells under different developmental controls and patterns of gene expression. With this in mind, SAGE was used to investigate the gene expression profile during xylogenesis in the cambial region of 1-3 year old *E. grandis* trees. 385 clones were sequenced producing 13,137 SAGE tags of which 5199 were singletons. Preliminary analysis using Brazilian databases identified 400 genes with only 4 tags not found in the database. A functional analysis of these identified genes was carried out creating 6 functional classes. The most abundant class 22.7% contained genes involved in energy production and cellular maintenance. 17.9% represented the second most abundant class containing genes involved in cellular regulation, developmental processes and stress and disease responses. Approximately 30 transcripts are directly involved in cell wall metabolism, including a chitinase-like protein involved in the metabolism of secondary cell walls. 26.8% of the annotated tags correspond to mRNAs with undefined functions. In parallel, two other SAGE libraries were constructed using material from two different developmental stages (1-6 months and 6 years) and another 200 clones will be sequenced. Analyses of these three SAGE libraries will allow the identification of the differential gene expression patterns present at each developmental stage, thereby demonstrating the principal processes responsible for the formation of wood. Besides this, analysis of the proteins produced at these developmental stages is also being carried out producing complementary data allowing in depth analysis of the processes involved in wood production.

S1.11p

SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) IN YOUNG *EUCALYPTUS GRANDIS* PLANT STEMS

Raphael T. Carneiro[1], Mayra C.C.G. de Carvalho[1], Danielle G. Gomes[1], Guillermo R. Salvatierra[1], David H. Moon[1], Shinitiro Oda[2], Carlos A. Labate[1]

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Eucalyptus species present excellent potential for wood production because of their diversity, adaptability high yield and physico-mechanical properties of the raw material permit numerous utilizations, principally for the production of paper and cellulose. World trade figure put wood as the fifth most important product, with Brazil becoming the main producer of cellulose using *Eucalyptus* as the raw material. Xylogenesis represents an ideal model for the study of cellular differentiation, the process being directed by the coordinated expression of numerous genes with structural and regulatory functions (many of which are unknown). A further complicating factor is that there are many different stages that contribute to xylem formation, cellular division and expansion, biosynthesis, accumulation and deposition of monosaccharides, condensation of the cell wall, lignification and programmed cell death. In this study SAGE was used to establish an expression profile of the genes involved in xylogenesis in young (1-6 months old) *E. grandis* stems. Currently 367 clones were sequenced producing 11,045 tags, 4,946 singletons. Another 200 clones are programmed for sequencing. The 415 tags most expressed were identified using Brazilian databases (NCBI e Forests) and used to be separated into functional categories. It was possible to observe that the category containing genes involved in energy production and cellular maintenance was the most highly expressed ~23% and the category containing genes involved in growth, development and cellular regulation was the second most highly expressed ~18%. Of the annotated genes ~25,8% corresponded to genes that were hypothetically expressed or without homology. This study is part of a larger project being developed within the laboratory that involved the production of two other libraries from the cambial tissue of *E. grandis* with different ages (1-3 years and 6 years old). Finally the data for the three libraries will be analysed together to identify differentially expressed genes during the different developmental stages studied. In parallel protein studies (Proteome) are being carried out on the same experimental samples permitting the identification of expression patterns of the genes involved in the formation and quality of wood from *E. grandis* destined for cellulose and paper the production.

S1.12p

IDENTIFICATION, ANALYSIS AND SEQUENCING OF PROTEINS RESPONSIBLE FOR THE PROCESS OF WOOD FORMATION IN *EUCALYPTUS GRANDIS* UNDER DIFFERENT GROWTH PHASES

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The process of wood formation is a crucial economical factor for the forestry industry and it is also of ecological importance, although little is known about protein responsible for wood formation in wood species. The identification analysis and sequencing of proteins provides such information of wood formation. Using proteomics methodologies such as HPLC and mass spectrometry we have started the proteome analysis of *Eucalyptus grandis* under different growth phases. Wood powdered tissue in liquid nitrogen was homogenized with 10%TCA and 0.07% ß-mercaptoethanol in acetone. Proteins were precipitated for 1h at -20ºC. After centrifugation at 15000g for 15 min, the protein pellets were rinsed with acetone containing 0.07% ß-mercaptoethanol for 1h at –20ºC. The supernatant was removed and protein pellet vacuum-dried and resuspended in 10µl/mg of solubilization buffer (7M urea, 2M thiourea, 4% Chaps, 10mM DTT). The separation of proteins was performed with combination of IEX in first-dimension and RPC in the second dimension. The fractions were dried in a speed vac and digested overnight at 37ºC with trypsin at ratio of 1:50(w/w). The reaction was stopped by adding formic acid (5%). The peptide samples were separated and analyzed using a Q-TOF API (Micromass) LC-MS/MS system. The peptides were separated in C18, in buffer A (0.1% formic acid, 5% ACN in water) by a 45 min linear gradient of 0-80% buffer B (0.1% formic acid, 5% water in 95% ACN). The MS/MS was searched against the NCBI database using BioLynx (Micromass). The method have provide a faster and accurate tool for separation and identify of protein which are differentially expressed under different growth phases of *Eucalyptus grandis*.

S1.13p

PROTEOMIC ANALYSIS OF THE CAMBIAL REGION OF *EUCALYPTUS GRANDIS*: UNDERSTANDING THE METABOLIC ROUTES AND WOOD QUALITY DURING DEVELOPMENT

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Two databases, transcriptome and proteome, were constructed using cambial material from *E. grandis* in order to search for genes involved in wood quality. Genomic expression data were obtained using SAGE and protein sequencing using mass spectrometry (Q-TOF, Micromass) on a LC/MSMS platform. The results show different patterns of protein expression during stem development (three different ages from seedlings to 6 years old) of *E. grandis* using two-dimensional gels. One bulk from the cambial material of a half sibling family was made from each age. This work presents the protein profile from the 3 years old bulk. Comparisons between the protein and the corresponding SAGE data, obtained from the same tissues, were used to confirm the identity of genes responsible for their expression. Protein sequencing data were analyzed using international databases and the SAGE database was used to determine the expression levels of the relevant genes. With the integration of these two databases, Transcriptome and Proteome, it is possible to identify candidate genes for the manipulation of wood quality, and to understand post-transcriptional and post-translational processes.

S1.14p

CHARACTERISATION OF THE PROTEOME FROM *EUCALYPTUS GRANDIS* CAMBIAL TISSUE

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Brazil maintains an area of approximately 3 million hectares reforested with *Eucalyptus* to attend the necessities of diverse forestry sectors. The perspective of conquering new markets is promising, promoting an ever-increasing investment in genetic research to alter eucalyptus wood quality. The major interest is directed to the genes involved in secondary wood growth from the vascular cambium. Understanding cambium differentiation is essential because tree growth is directly affected by the continuous development of secondary phloem and xylem. Until recently it was thought that the only way to achieve these objectives was to use genomic strategies. Due to the availability of large quantities of EST sequences, coupled to the development of better analytical methods for the characterization of proteins, proteome was transformed into the largest research field in functional genomics. The objective of the present study is to characterize the proteome of the cambial tissue from 6 year old *Eucalyptus grandis* trees. Proteins were extracted from a bulk made from 30 individuals and resolved on two-dimensional gels. The experimental strategy involved the use of liquid chromatography coupled to mass spectrometry ESI Q-TOF (electrospray ionization quadrupole time-of-flight) to obtain the amino acid sequences of peptides generated by tryptic digests. The sequence is analysed using protein databases (NCBI, SwissProt) to identify and determine the biological function of the protein. This study is part of a larger project to investigate the proteome and transcriptome of the cambial tissue from *Eucalyptus* trees at three developmental stages; 0-6 months, 1-3 years, 5-6 years, in order to further understand the molecular and genetic regulation of xylogenesis during the productive growth cycle of the tree.

S1.15p

THE USE OF SAGE TECHNOLOGY TO INVESTIGATE GENE EXPRESSION PATTERNS AFFECTING THE ECONOMICALLY IMPORTANT PHENOTYPE, WOOD QUALITY, IN *EUCALYPTUS* SPP

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An important factor in competitiveness of the cellulose and paper industry is the selection of trees that form homogenous plantations facilitating the production of cellulose pulp with a high industrial yield with low costs. In Brazil, forest improvement programs by the pulp and paper industries are highlighted by the excellent results obtained in the area of cloning *Eucalyptus spp*, national industries have already reached 60 m3/ha/year. The object of this study was to investigate gene expression in the cambial region at various stages in the productive cycle of *Eucalyptus* trees. During the summer, the growth rate of *Eucalyptus* trees is greater and consequently the activity of the cambial cells would be higher, the object of our study. The tissue from the cambial region of 35 individual *Eucalyptus grandis* clones were sampled for each age. Normally the productive cycle is 7 years in Brazil, there for three ages were chosen to represent the whole cycle: 1-6 months, 1-3 years and 5-7 years. Preliminary sequencing generated 11.283 13.313 and 13.172 for the 1-6 month, 1-3 year and 5-7 year libraries, respectively. Analysis of the identified genes indicated that an average from the three libraries show that 20.5% for the genes were involved in primary and secondary metabolism, 16.7% in cell regulation, disease and stress responses, 5.9% in transport, 12.9% in the formation of cellular structures, 12.7% in macromolecular metabolism and 28.7% without classification.

S1.16p

ISOLATION AND EXPRESSION ANALYSIS OF 2465 COLD-REGULATED ESTS FROM *EUCALYPTUS*

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Due to fast growth and fiber quality, *Eucalyptus* is very important for the paper industry. However, being an evergreen woody plant without endodormancy, this species is particularly exposed to cold. Although widely distributed across the world, its extension is mostly restricted to southern areas by freezing sensitivity. Its survival during a frost strongly depends on its constitutive tolerance at the cell level and also on its acclimation capacity. Based on a functional genomics approach of cold response in *Eucalyptus*, the project is targeting a double objective:

- Better understanding of molecular mechanisms involved in cold acclimation of *Eucalyptus* including the protective response and its regulation.
- Identification of reliable molecular markers (SNPs) of cold tolerance for MAS using a candidate gene approach. As a first step towards these goals was the isolation and first characterization of interesting target genes involved in cold acclimation for further genetic and functional studies. Two mains experimental phases were involved: after isolation of a pool of cold-regulated genes the molecular and expressional characterization of chosen genes was performed. For the isolation of cold-regulated genes, 3 cDNA libraries were constructed containing a total of 300 000 ESTs and corresponding to 2 acclimation subtractive libraries from cell suspension cultures and a complete cDNA library from leaves of cold acclimated plants. Among the 3468 analysed genes, 71% are differentially expressed after a chilling exposure and this high representation of cold regulated genes is confirmed by sequence analysis. Presence of transcription factor genes and in particular CBF and its regulon as well as new genes validated this set of genes as a very powerful tool for our further studies. Beyond the description of the main functional categories of these cold-regulated genes, it will be presented here the gene clustering from macroarray expression analysis according to stress response specificity and kinetics, particularly during *Eucalyptus* cold acclimation.

S1.17p

GENES POTENTIALLY INFLUENCING PULP YIELD IN *EUCALYPTUS NITENS*

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Increasingly, eucalypt breeding is being focussed on the development of breeds and clones with superior wood and pulp traits. Many of these traits are under moderate to strong genetic control and DNA markers have been used to identify QTL regions of the eucalypt genome that influence these traits. Linkage disequilibrium (LD) or association mapping using natural populations is useful for identify allelic variants in genes controlling traits of interest. We are identifying genes involved in fibre development and in particular genes affecting pulp yield. DNA microarrays containing ~5500 young xylem cDNAs were screened with probes synthesised from RNA isolated from trees with either high or low pulp yield. Fifty-six transcripts were differentially regulated, of which 29 were more abundant in high pulp trees and 26 more abundant in low pulp yield trees. All differentially expressed cDNAs were partially sequenced and searched against existing gene databases. Six genes were identified as putative pulp yield candidate genes based on their significant similarity to genes with known function, including a cellulose synthase, homeodomain-leucine zipper protein, no apical meristem (NAM) family protein, xyloglucan:xyloglucosyl transferase, beta-galactokinase and a zinc finger family protein. Real-Time PCR is being carried out to confirm the microarray results. We are identifying SNPs in the cellulose synthase and NAM genes using a panel of 16 individuals from natural populations of *E. nitens*. LD mapping will be conducted to identify alleles or haplotypes associated with a range of wood quality traits, including pulp yield, in an *E. nitens* association population of 300 trees.

S1.18p

TRANSCRIPT PROFILING TO STUDY WOOD FORMATION IN *EUCALYPTUS*

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The genus *Eucalyptus* includes the most widely used tree species for industrial plantation mainly for pulp making. As part of a Southern European* project aimed at implementing marker-assisted selection in *Eucalyptus* breeding programs, we are developing large scale EST sequencing programmes and wood transcriptome analyses. As a first step to identify major genes involved in wood formation, we have developed a reference cDNA macroarray of 231 *Eucalyptus* xylem unigenes preferentially and/or specifically expressed in xylem (Paux et al, 2004). We used this tool to carry out transcript profiling of these genes during a kinetics of tension wood formation. We investigated the expression patterns of the arrayed genes between tension, opposite and normal woods within the early stages of a tension time course (from 6h to one week after application of artificial bending of field-grown trees, Paux et al, 2005). This systematic gene expression profiling during tension wood formation provided new clues into the transcriptional regulatory network of genes preferentially expressed in xylem, but also highlighted the power of transcriptome analysis to select candidate genes responsible for the genetic and environmentally induced variation of wood quality traits. These results prompted us to make new SSH libraries using contrasted wood samples to enrich the array in new xylem-preferentially expressed genes. Expression data obtained with this second generation array will be discussed with regards to selection of major players in wood formation.

*Project joining academic (CIRAD, CNRS) and industrial partners (RAIZ, ENCE)

Paux et al, (2005) Transcript profiling of Eucalyptus xylem genes during tension wood formation. New Phytologist, 167, 89-100- Paux et al, (2004) Identification of genes preferentially expressed during wood formation in *Eucalyptus*. Plant Molecular Biology, 55:263-280

S1.19p

MAP-PING OUT TISSUE SPECIFIC EXPRESSION PROFILES OF POPLAR MAP KINASES AND MAP KINASE KINASES

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Plant mitogen-activated protein kinase (MAPK) cascades are composed of three interlinked protein kinases, namely MAPKKK, MAPKK, and MAPK. These modules amplify and rapidly transduce extracellular signals into various appropriate intracellular responses. MAPKKs and MAPKs are proteins that are activated via phosphorylation, therefore extensive work has been conducted at the post-translational level. On the other hand, few studies have investigated transcriptional regulation of these genes. Based on full sequencing of the poplar genome, we have assembled eight putative poplar MAPKKs (PoptrMKKs) and 21 putative poplar MAPKs (PoptrMPKs). Phylogenic analysis including all those genes as well as other plant MAPK/KK (e.g. *Arabidopsis* and rice) reveals a high level of correspondence among different groups in both families with a marked pattern of recent gene duplication in poplar. Using primers directed at the end terminal untranslated region, and real-time quantitative PCR, we have evaluated expression profiles of all members of these two gene families throughout 17 poplar tissues. We found that most PoptrMKKs and all PoptrMPKs are expressed differentially in the studied tissues. We also discovered that members of gene pairs originating from recent duplication events have different patterns of expression, consistent with the rapid evolution of specialized functions despite their high level of identity. In particular, transcripts for some genes become undetectable at specific stages of reproductive tissue development. Our analysis provides the most complete survey of MAPKK and MAPK expression profiles reported to date for a higher plant.

S1.20p

BIOINFORMATICS PLATFORMS IN THE GENOLYPTUS PROJECT

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Large scale sequencing projects demand a highly coordinated computational setup in order to store, process and analyze the large volume of raw data generated, a problem inevitably faced by several genome projects. Specifically, the Brazilian Network of *Eucalyptus* genome Research, GENOLYPTUS project has the particularity of generating sequencing data from several sources, namely: Expressed Sequence Tags (EST) from dozen of non-normalized libraries derived from different tissues and different species including *E. grandis, E. globulus, E. pellita* and *E. urophylla*; random genomic shotgun clones; Sequence-tagged connectors (BAC ends), BAC shotgun reads for selected genes and resequencing reads for SNP validation. Due to this intrinsic complexity, the already expected and increasingly greater demand for bioinformatics support as well as for safe long term data mirroring, storage and access within the GENOLYPTUS project, two physically separated but synchronized bioinformatics service centers were developed. At the Genomic Science Lab of the Catholic University of Brasilia a new software platform based on distributed object technology that concurrently manages several multi-laboratory sequencing projects in a portable way. Several services are offered ranging from data submission to automatic functional annotation, including classification into gene ontology (GO) classes, metabolic pathways and identification of conserved protein domains. Additionally, there are statistical routines for the assessment of differential expression between EST libraries, and genetic marker development through the identification of microsatellites and single nucleotide polymorphisms (SNPs). Using this system it was possible to probe for putatively differentially expressed genes between xylem and mature leaves, different biotic and abiotic stresses, and across the xylem of the various species. Moreover, different data mining services were provided for new microsatellite marker development both from ESTs and BAC ends as well as eSNP discovery for genotyping oligoarray construction. The second center was set up at the Genomics and Expresison Laboratory at Unicamp, the State University of Campinas, São Paulo, a lab that participated in several Brazilian genome sequencing efforts. In this center the researchers are able to analyze the sequences immediately after submission with the purpose of mining, shortening the time from the discovery of an important sequence and the wet-lab work. As the GENOLYPTUS project moves forward, new challenging tasks will arise. BAC fingerprinting data and BAC contig assembly in specific regions of the genome are starting to be generated at the same time that they are anchored to the genetic maps with microsatellites. Genetic marker and QTL mapping data coming from multiple pedigrees is also pouring in and soon other sources such as microarray data will also be generated. The analysis, integration and organization of all these sources of genomic information are not an easy undertaking. It will require continued effort to effectively deliver the platform of genomic resources that constitute the main objective of the GENOLYPTUS project.

Financial support: Genolyptus project (Ministry of Science and Technology and group of supporting forest based companies).

S1.21p

SEQUENCING OF THE *EUCALYPTUS* TRANSCRIPTOME IN THE GENOLYPTUS PROJECT

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The main goals of the Brazilian Network of *Eucalyptus* Genome Research (GENOLYPTUS) include the discovery, sequencing and mapping of economically important genes and the determination of their activities in different species of *Eucalyptus*, aiming at the incorporation of new genomic technologies into advanced eucalypt breeding programs and forest production. The subproject "Sequencing of the *Eucalyptus* Transcriptome" entitles the sequencing effort to achieve 150,000 Expressed sequence tags (EST) from different expression libraries. The objective is the identification of all ~30,000 estimated eucalypt genes, with special emphasis on those involved in wood formation. More than 20 libraries were constructed from different organs, tissues and whole seedlings submitted to several treatments derived from four *Eucalyptus* species. Approximately 120,000 ESTs were produced, analyzed, validated and arranged into ~34,000 clusters and singlets based on sequence homology. The putative functions of the sequenced clones were attributed automatically by BLAST search. A more detailed analysis of ESTs belonging to libraries of *E. grandis* treated (TS) and untreated (SE) seedlings was performed. ESTs were grouped according to their putative biological function and those with "no hit", "unknown function" or "hypothetical/putative function" represented 60 percent of the total. We have selected *in silico* the 500 most differentially expressed genes between TS x SE and organized samples for macroarray experiments. After the confirmation of the electronic northern by gene expression experiments, we plan to proceed with the genetic and biochemical characterization of these genes in transgenic plants.

Financial support: Genolyptus project (Ministry of Science and Technology and group of supporting forest based companies).

S1.22p

SEQUENCING AND DIFFERENTIAL EXPRESSION OF XYLEM SPECIFIC GENES FROM TWO *EUCALYPTUS* SPECIES WITH HIGHLY CONTRASTING WOOD PROPERTIES

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We have sequenced and validated approximately 15,300 and 9,200 ESTs derived from cDNA libraries of vascular tissues from *Eucalyptus globulus* and *E. pellita* respectively. These species have highly contrasting wood properties, the first being considered a high quality cellulose pulp yielding species. A digital differential display approach was employed to select the 500 clusters with the most contrasting expression levels, in order to perform an initial screening for genes responsible for the observed differences in wood quality. Sequences were annotated by their function and classified using the Gene Ontology parameters. Eighteen clusters with distinct cellular functions were considered constitutive; therefore they were used to normalize the relative expression levels between libraries. Among the 500 selected genes, 41 were not represented in GenBank, despite of their relative high expression. The absolute values for reads presence ranged from 0 to 52, with relative expression levels ranging from 1,9 to 38, suggesting possible important roles for these novel genes. Among the identified genes, we found relative expression levels ranging from 140 to 0 when ranking *E. globulus* versus *E. pellita* genes, and 58 to 0 when ranking *E. pellita* versus *E. globulus* genes. Several genes involved in lignin and cellulose biosynthesis were found as well as transcription factors. Numerous genes involved in protein folding, especially smHSPs were identified in the *E. globulus* library. Conversely, genes involved in oxidative stresses were detected in the *E. pellita* library. Arrays and real time PCR assays are being conducted in order to confirm the electronic results.

Financial support: Genolyptus project (Ministry of Science and Technology and group of supporting forest based companies).

S2. Tree interactions with pests, pathogens and symbionts

S2.1

GENOME EXPLORATION IN THE DUTCH ELM DISEASE FUNGUS, *OPHIOSTOMA NOVO-ULMI*

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The ascomycete fungus *Ophiostoma novo-ulmi* is the aggressive pathogen responsible for the current pandemic of Dutch Elm Disease. The mechanisms by which the fungus kills its host remain unexplained. We are combining genetic, molecular and genomic approaches to investigate the nuclear genome of *O. novo-ulmi* and identify and characterize genes controlling parasitic fitness in this organism. We have mapped over 150 genetic markers on 11 linkage groups representing 7 chromosomes, which can be resolved by pulsed-field gel electrophoresis. Mendelian analysis of progeny from controlled crosses involving a natural variant with unusually low aggressiveness has allowed us to identify and localize a putative pathogenicity locus, named *Pat1*. Chromosome walking from two RAPD loci linked to *Pat1* led us to identify candidate genes, including a gene sharing high sequence similarity with the Mep/Amt gene family of ammonium transporters. Disruption and overexpression studies of these genes are under way. We have also used insertional mutagenesis (REMI) to obtain additional *O. novo-ulmi* mutants with altered pathogenicity. Three insertional mutants with significantly lower pathogenicity towards two-year-old *Ulmus parvifolia x U. americana* saplings were obtained and are being investigated further. Finally, through a collaborative effort with three other research teams, we initiated a large-scale genomic study of *O. novo-ulmi*. An expressed sequence tag (EST) reference library from yeast cells was established: it contains 4386 readable sequences with an average trimmed length of 500 bp. Smaller, more specific EST libraries were prepared using suppression subtractive hybridization (SSH). These include libraries from mycelium grown at suboptimal temperatures, from fruiting bodies, or from yeast cells inoculated to calli of highly susceptible *U. americana*. Macroarray analysis is currently underway for the detection of differentially expressed genes which we intend to characterize further.

S2.2

RECENT STUDIES IN THE CONIFER - *HETEROBASIDION* PATHOSYSTEM

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Root rot caused by the basidiomycete *Heterobasidion annosum* s.l. is one of the most destructive diseases of conifers in the northern boreal and temperate regions of the world. The fungus consists of three European intersterile subspecies P (*H. annosum*), S (*H. parviporum*) and F (*H. abietinum*) with differing main host preferences; pine, spruce, and fir, respectively. In North America, two intersterile groups are present, P and S/F, but these have not yet been given scientific names. About 4000 ESTs were collected from *H. annosum* growing in contact with its host. About 70 % of the genes identified showed high similarities to known proteins and 16 % had similarity only with proteins with unknown functions. Detailed expression studies confirm up-regulation *in planta* of putative pathogenicity factors e.g. toxin production, cell wall degrading enzymes and proteins known to be involved in oxidative stress. Recently, an AFLP-based genetic linkage map was established that allowed for mapping QTLs for pathogenic growth towards seedling roots and pine phloem. The next step underway is to verify the identity of candidate genes located within the established region of the genome. Future functional analysis of both QTL and EST-derived candidate genes will be aided by the recently established *Agrobacterium*-mediated transformation system in *Heterobasidion*. Host responses to the pathogen have been studied using microarrays of *Pinus taeda* genes. Up-regulation of phenyl propanoid and stilbene pathways as well as antimicrobial protein production was detected within 5 and 15 days of infection. The antimicrobial protein was located in the cell wall according to immuno gold labelling. Infection experiments on seedling material have shown that resistance to *H. annosum* has a relatively high heritability. Work is in progress to identify genes involved, with the long term goal to guide resistance breeding.

S2.3

TRANSCRIPTIONAL PROFILE OF THE METABOLIC RESPONSE OF *EUCALYPTUS GRANDIS* TO THE FUNGAL PATHOGEN *PUCCINIA PSIDII*

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The Forest activities represent around 4% of the Brazilian Gross Domestic Product with *Eucalyptus* being the main species used for commercial reforestation. *Eucalyptus* is an important renewable resource for many reasons including rapid growth, diversity and low production costs that provide the raw material for many products, principally cellulose pulp, paper and charcoal. However, productivity is affected by neotropical rust, caused by the biotrophic fungus *Puccinia psidii* Winter. Rust management normally involves the use of fungicides but the use of natural resistance is a much better alternative due to lower costs and minimal ambiental impact. Although natural resistance is known the actual molecular mechanisms involved are poorly understood in *Eucalyptus*. SAGE "Serial Analysis of Gene Expression" is a quantitative genome-wide method for obtaining gene expression profiles of known and unknown genes. The aim of this study is to obtain an expression profile of the genes activated during fungal infection. Two contrasting SAGE libraries were constructed; one using the total RNA from a bulk of resistant and the other from sensitive individuals. Preliminary sequencing generated 13885 and 12121 tags in the resistant and sensitive libraries, respectively. Our results indicate the presence of 1769 genes represented by 3 or more tags, with 359 differentially expressed transcripts, of which 125 are exclusively expressed in the resistant phenotype. Of the genes identified using international (NCBI and Pfam) databases, differentially expressed genes were more likely to be from the categories involved in energy production, cellular maintenance and transport, whereas those involved in cellular regulation and differentiation, stress, macromolecular metabolism and the formation of cellular structures are more likely to be exclusively expressed in the resistant phenotype. The sequencing of more clones is programmed with the aim of decreasing the number of singletons present and increasing the statistical significance of the genes expressed at the lowest levels. Genes exclusively expressed in the resistant phenotype will serve as candidates, not only for use in marker assisted improvement programs, but also as candidates for future transformation experiments.

S2.4

DEFENSE REACTIONS IN NORWAY SPRUCE TOWARD THE PATHOGENIC ROOT-ROT CAUSING FUNGUS *HETEROBASIDION ANNOSUM*

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The root-rot causing fungus *Heterobasidion annosum* can attack both spruce and pine trees and is the economically most damaging pathogen in northern European forestry. We have monitored the *Heterobasidion annosum* S-type (recently named *H. Parviporum*) colonization rate and expression of host chitinases and other host transcripts in Norway spruce material with differing resistances. Transcript levels of three chitinases, representing classes I, II and IV, were monitored with real-time PCR. Ramets of two 33 -year-old clones differing in resistance were employed as host material and inoculation and wounding was performed. Multiplex real-time PCR detection of host and pathogen DNA was also performed to follow the colonization of the host tissues by the pathogen and the collapse in host DNA levels in infected regions. Host defence transcript levels, as an indicator of the host defence response, were monitored with singleplex real-time PCR. Three days after inoculation, comparable colonization levels were observed in both clones in the area immediately adjacent to inoculation. Fourteen days after infection, pathogen colonization was restricted to the area immediately adjacent to the site of inoculation for the strong clone (589), but had progressed further into the host tissue in the weak clone (409). Transcript levels of the class II and IV chitinases increased following wounding or inoculation, while the transcript level of the class I chitinase declined following these treatments. Transcript levels of the class II and class IV chitinases were higher in areas immediately adjacent to the inoculation site in 589 than in similar sites in 409 three days after inoculation, suggesting that the clones differ in the rate of pathogen perception and host defence signal transduction. This and earlier experiments using mature spruce clones as substrate indicate that it is the speed of the host response and not maximum amplitude of the host response that is the most crucial component in an efficient defence in Norway spruce toward pathogenic fungi such as *H. annosum*.

S2.5

REGULATION OF CARBON TRANSFER IN THE MISTLETOE-HOST (POPLAR) INTERFACE

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Viscum album L., the European mistletoe, is a xylem-parasitic higher plant growing on trees. Due to its green leaves and stems it was assumed to be carbon autotrophic. Recent studies, however, show that mistletoe plants can make use of organic carbon supplied by the host (poplar) xylem. Our studies with tissues of the host/parasite interface (sinker and surrounding host tissue) indicate that the parasite can even regulate sugar concentrations in the host xylem. In infected poplar branches these showed two maxima during the year: one in early spring (bud swelling of the host) and a second during early summer (growth of the mistletoe). In the mistletoe – host interaction zone, sucrose transported in the host xylem seems to be cleaved by induction of cell wall invertases. Monosaccharides formed and released into an apoplastic continuum are taken up by the mistletoe and are transiently stored there as starch. The aim of our work is to locate the induction of cell wall invertases and other sucrose metabolising enzymes in the host or parasite.

S2.6

SCREENING AND SELECTION OF HALF-SIB FAMILIES OF *EUCALYPTUS SMITHII* FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI* AND *P. NICOTIANAE*

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Phytophthora root and collar rot is a serious disease problem associated with cold tolerant eucalypts that are commonly planted in high altitude areas of South Africa. One of these species, *Eucalyptus smithii*, displays rapid growth and excellent pulping properties. However, this species has high mortality particularly at establishment. This problem has been attributed to the root rot disease complex involving *Phytophthora cinnamomi* and *P. nicotianae*. The aim of this study was to screen and select *E. smithii* families with tolerance to *Phytophthora* root and collar rot. Twelve half-sib families of *E. smithii* were screened for tolerance to *Phytophthora* dieback in a greenhouse. *E. smithii* stems were inoculated with virulent isolates of *P. cinnamomi* and *P. nicotianae* and resulting lesion lengths were measured after three weeks. A completely randomised block designed experiment with 10 replicates per isolate and a control was used. Families were rated as tolerant or susceptible according to the resulting lesion lengths. Three highly tolerant and susceptible *E. smithii* half-sib families were identified using the stem inoculation technique. Results of the greenhouse study were closely correlated with susceptibility of families in the field. Our results indicate that stem inoculations of young trees in the greenhouse could be used to determine tolerance to *Phytophthora* root and collar rot in *E. smithii* planting stock.

50 half-sib families

Lignome project ?

S2.7

INTERACTING GENOMES IN TREE-PEST RESPONSE

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With their long life cycle, trees must have developed distinct defence mechanisms allowing long-term survival. Moreover, gene expression is altered in both plant and pathogen upon interaction with each other. Poplar is a fast growing species found worldwide and is economically important. Unfortunately poplar is susceptible to several forest pests including poplar leaf rust caused by *Melampsora* species. We propose a genomics approach to identify fungal and plant genes that are up- or down-regulated in interactions involving trees and biotrophic rust. Gene expression will be monitored during compatible, incompatible and non-host interactions resulting from the infection of different poplar clones with *Melampsora* species. More specifically, poplar genes encoding for transcription factors (WRKY and TGA) as well as genes belonging to the signalling cascade (MAP kinase, NPR1) linked with those transcription factors have been selected. Experiments on gain of function and loss of function of those candidate genes have been undertaken. We will present the molecular analysis of candidate genes, phylogenetic relationship and, results on transcript profiling and functional analysis.

S2.8

STUDIES OF SIGNALING PATHWAYS IN RESPONSE TO HYPAPHORINE AND TO HYPAPHORINE/IAA COMPETITIVE INTERACTION IN *EUCALYPTUS* AND POPLAR ROOTS

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Ectomycorrhizal ontogenesis implies changes both in fungi with formation of a mantle and a Hartig net and in roots with stimulation of lateral root initiation, arrest of root hairs elongation and in some species, radial elongation of root cortical cells. Such morphogenetic changes require mutual recognition and exchange of signalling molecules between the two partners. Hypaphorine, a major indole alkaloid compound was purified in fungus *Pisolithus microcarpus* mycelium. Since, the role of hypaphorine in the first steps of the *Eucalyptus globulus / Pisolithus microcarpus* symbiosis has been widely investigated. Root treatment with hypaphorine induced a rapid decrease in the growth rate both of Poplar and of *Eucalyptus* root hairs. The anti-indole-3-acetic acid (IAA)-antagonist activity of this molecule has been also recognized. Evidence of this competitive antagonism includes gene expression, molecule–molecule interaction, primary root growth and root hair elongation. The objective of our present study is to examine signalling pathways involved in response to hypaphorine and to hypaphorine/IAA competitive antagonism in Poplar and *Eucalyptus* root. We firstly focussed our studies on events playing crucial roles in root hairs as calcium, actin and vesicle trafficking. Visualisation of filamentous actin in *Eucalyptus* root hairs clearly showed that hypaphorine modifies actin cytoskeleton dynamism in root hair. Using a calcium sensitive-dye, we revealed that hypaphorine supply leads to a reduction of calcium gradient, previously observed in control growing root hairs. Hypaphorine and its interaction with IAA also controlled the dynamic of membrane trafficking in *Eucalyptus* root hairs. A wider investigation, using a poplar micro array (I.E a Unigene of 4608 cDNAs of *Populus trichocarpa X deltoides*) allowed us to discriminate between a few new hypaphorine-regulated genes among a vast majority of genes whose expression is not affected by hypaphorine treatment. An extensin-like protein coding gene, involved in cell wall plasticity, is down-regulated by hypaphorine and up-regulated by IAA. With all together, these recent results suggest that hypaphorine and the hypaphorine/IAA interaction act firstly on an upstream event controlling root hair elongation during ectomycorrhizal ontogenesis. Regulation of transcription of extensin-coding genes by hypaphorine and IAA highly supports evidence of the role of IAA/hypaphorine competitive interaction during the establishment of ectomycorrhizae by controlling cell plasticity and cell elongation.

S2.9p

BEECH LEAF COLONIZATION BY THE ENDOPHYTE *APIOGNOMONIA ERRABUNDA* DRAMATICALLY DEPENDS ON LIGHT EXPOSURE AND CLIMATIC CONDITIONS

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Ozone and light effects on endophytic colonization by *Apiognomonia errabunda* of adult beech trees (*Fagus sylvatica*) and their putative mediation by internal defence compounds were studied at the Kranzberg Forest free-air ozone fumigation site. *A. errabunda* colonization was quantified by "Real-time PCR" (QPCR). *A. errabunda*-specific primers allowed detection without interference by DNA from European beech and several species of common plant pathogenic fungi like *Mycosphaerella, Alternaria, Botrytis*, and *Fusarium*. Colonization levels of sun and shade leaves of European beech trees exposed either to ambient or twice ambient ozone regimes were determined. Colonization was significantly higher in shade compared to sun leaves. Ozone exhibited a small but significant inhibitory effect on fungal colonization only in young sun and shade leaves. The hot and dry summer of 2003 reduced fungal colonization dramatically, more pronounced than did ozone treatment or sun exposure. Levels of soluble and cell wall-bound phenolic compounds were approximately twice as high in sun than in shade leaves. Acylated flavonol 3-*O*-glycosides with putatively high UV-B shielding effect were very low in shade canopy leaves. Ozone had only a minor influence on secondary metabolites in sun leaves. It slightly increased kaempferol 3-*O*-glucoside levels exclusively in shade leaves. The prominent hydroxycinnamic acid derivative, chlorogenic acid, was tested for its growth-inhibiting activity against *Apiognomonia* and showed an IC50 of approximately 8 mM. Appearance of *Apiognomonia*-related necroses strongly correlated with the occurrence of the stress metabolite, 3,3',4,4'-tetramethoxybiphenyl. Infection success of *Apiognomonia* highly depended on light exposure, presumably affected by the endogenous levels of constitutive phenolic compounds. Ozone exerted only minor, modulating effects, whereas climatic factors such as pronounced heat periods and drought were dramatically overriding.

S2.10p

PRELIMINARY POPULATION GENETIC STUDIES ON THE *EUCALYPTUS* STEM CANKER FUNGUS *CONIOTHYRIUM ZULUENSE*

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Canker of *Eucalyptus* caused by the fungal pathogen *Coniothyrium zuluense* is a relatively new disease of plantation-grown *Eucalyptus*, particularly in tropical and sub-tropical areas of Africa, South America and South East Asia. The first signs of infection due to this pathogen were observed in the late 1980's in South Africa. In the mid 1990's, the fungus was isolated from *Eucalyptus* stem lesions and described as *Coniothyrium zuluense*. Recently, the taxonomic status of the fungus has been re-considered and the fungus was recognised as a species of *Colletogloeopsis*, an anamorph of *Mycosphaerella*. Very little is known about the biology or epidemiology of *C. zuluense*. It is hypothesized that the pathogen has been introduced into new environments through infected *Eucalyptus* seeds, and possibly that South Africa has been a source of infection for other countries. The aim of this investigation was to develop microsatellite markers in order to consider the possible origin of *C. zuluense*. A second aim was to establish whether the absence of teleomorph structures indicates that *C. zuluense* is a purely asexual species. In order to accomplish these objectives, nine microsatellite markers were developed using the FIASCO enrichment method. Preliminary results show that the view of South Africa as the original source of *C. zuluense* is not correct. Furthermore, it appears that a sexual state probably occurs for the fungus, at least in the South African population. These studies together with future coalescent analyses are likely to provide a considerably better understanding of the epidemiology and dispersal mechanisms of *C. zuluense*.

S2.11p

MICROSATELLITE MARKERS REVEAL GENETIC DIVERSITY IN SOUTHERN HEMISPHERE POPULATIONS OF *DOTHISTROMA SEPTOSPORUM*

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Dothistroma septosporum has caused devastating losses to pine plantations in various Southern Hemisphere countries. The propagation of *P. radiata* has thus been discontinued in Kenya and Brazil due to the difficulties in controlling the disease. Surprisingly, *D. septosporum* has been present in South Africa since the mid-1960s but its distribution has been limited to the Eastern Cape Province with sporadic outbreaks on *P. radiata* and low disease incidence. Recently, however, the disease has been discovered in the Northern Province where damage has been substantial. In order to study the dynamics and gene diversities of *D. septosporum* populations in various parts of the world and to determine genetic relatedness in the fungus representing different populations, we have developed a set of 11 polymorphic microsatellite markers using both ISSR PCR and the FIASCO enrichment technique. The efficiency of these markers was tested on a limited population of isolates from Africa including two different provinces from RSA and one from Kenya. In the samples tested, 35 alleles across 13 markers were identified. All three populations contained unique alleles with Kenya having seven that are absent from the South African populations. Only 56% of the alleles present in South Africa are shared between the two provinces. From these preliminary data, we conclude that the South African populations are different from each other and from that of the Kenyan population. Each of these populations appears to have originated from separate introductions. Although the fungus appears to be fully asexual in the areas considered, the populations are also not clonal as has been suggested for the isolates in New Zealand. These preliminary results lead confidence to the usefulness of the developed primers that will now be used to compare populations of *D. septosporum* from different countries.

S2.12p

A NEW SPECIES OF *PSEUDOCERCOSPORA* FROM *EUCALYPTUS* LEAVES IN THAILAND

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Pseudocercospora is a large and morphologically variable genus that houses the anamorphs of many *Mycosphaerella* spp. A large number of *Pseudocercospora* spp. have been identified from *Eucalyptus* leaves where they cause leaf spots and often defoliation of trees. A very obvious leaf spot disease, particularly of *E. camaldulensis* and hybrids of this and other species, caused by a species of *Pseudocercosopra* is well known and often damaging in Thailand. The fungus is characterised by very obvious angular leaf spots with chlorotic margins and dark bunches of conidiophores on the underside of lesions. The aim of this study was to identify the *Pseudocercospora* sp. causing leaf spot outbreaks in Thailand. This was achieved using morphological studies and comparisons of DNA sequences for the ITS, EF-1 alpha and ACT gene regions. The emerging data were compared with those existing for *Pseudocercospora* spp. from *Eucalyptus*. Results of these studies lead us to conclude that, the *Pseudocercospora* sp. collected from *Eucalyptus* in Thailand represents a new species that is currently being described. The identification of this new *Pseudocercospora* sp. increases the already large number of these fungi known to occur on *Eucalyptus* and it also fortifies our ability to reduce the risk of unwanted introductions of *Eucalyptus* pathogens into new areas.

S2.13p

ROOT LIGNIN AND THE EFFECT OF LIGNIN MODIFICATION ON ECTOMYCORRHIZAL SYMBIOSIS BETWEEN SILVER BIRCH AND *PAXILLUS INVOLUTUS*

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Silver birch (*Betula pendula* Roth) is economically the most important deciduous tree species in Nordic countries, and the birch roundwood is raw material in the chemical pulp industry. Silver birch is the main broad-leaf species of conventional tree breeding, and it is also one of the key species in Nordic forest ecosystems. Biosynthesis and modification of birch wood lignin is currently studied, because lignin-modified wood may turn out to be a potential raw material for the pulp industry. Wood lignin modifications have, however, evoked concern about the potential effects on tree fitness and unintended side-effects and pleiotrophic effects of the transgenes having e.g. ecological impacts. We have produced transgenic silver birches by introducing the *COMT* (caffeate/5-hydroxyferulate O-methyltransferase) gene from *Populus tremuloides* L. in sense-orientation into birch clones A and E5396. The promoters used to drive the *PtCOMT* were CaMV 35S and UbB1-promoter from sunflower. In the 35S-PtCOMT lines, a reduced syringyl/guaiacyl (S/G) ratio of stem wood and leaf lignin has been found in comparison of UbB1-PtCOMT and non-modified control lines. The aim of the present study was to examine the root lignin content and composition in the transgenic silver birches carrying the *PtCOMT*, and furthermore, the ability of the COMT-modified birch lines to form symbiosis with an ectomycorrhizal fungus *Paxillus involutus* under in vitro conditions. In the accordance with the stem wood and leaf lignin observations, the introduction of 35S-PtCOMT into birch was found to result in remarkably reduced S/G ratio in root lignin too. The root lignin S/G ratios of all the *in vitro* birches, including the controls, were low when compared to greenhouse-grown plants, and were generally reduced slightly as a consequence of fungal inoculation. The presence of the fungus improved the viability of the birch plants, and all the birch lines, both transgenic and controls, were observed to form ectomycorrhizal symbiosis with *Paxillus involutus*. However, the number of lateral roots in the inoculated in vitro-plants, the number of lateral roots covered with fungal hyphae, and formation of Hartig net between epidermal cells varied depending on the birch line.

S2.14p

CHARACTERIZATION OF THE MATING-TYPE LOCI OF *AMYLOSTEREUM AREOLATUM*

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Amylostereum areolatum is a white-rot homobasidiomycetous fungus, which is effectively spread via their asexual arthrospores by the *Sirex* woodwasp. In combination the wasp and fungus represents a serious threat to pine-based forestry in the Southern Hemisphere. In its native environments, *A. areolatum* also reproduce sexually with a heterothallic and tetrapolar mating system. In this system, mating type compatibility is determined by two unlinked mating type loci (loci A and B), each of which consists of two linked sub-loci (A1, A2 and B1 and B2). Although the structure and function of these loci are well characterised in *Coprinus cinereus* and *Schizophyllum commune*, not much is known about the mating type loci of *A. areolatum*. The aim of this study was to use the information from these model homobasidiomycetes to isolate and characterize the B-mating type locus of *A. areolatum*. For this purpose we used degenerate primers based on the sequences for *C. cinereus* and *S. commune*. Even though these regions are hyperdiverse, we successfully identified sub-locus B2 using these primers. The remainder of B2 sub-locus was obtained through PCR-based genome walking. Sequence analysis revealed that sub-locus Bb of *A. areolatum* is similar to those of *C. cinereus* and *S. commune*, in harbouring genes encoding mating pheromones and pheromone receptors involved in the mating pheromone response pathway. These results suggest that sub-locus Ba and mating type locus A of *A. areolatum* may also be characterised in a similar fashion by using available information of the model homobasidiomycetes. In this way we hope to gain a better understanding of the processes that shape and govern the population biology and evolution of this important fungus.

S2.15p

DNA BASED DETECTION OF *FUSARIUM CIRCINATUM*, THE CAUSAL AGENT OF PITCH CANKER

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Pitch canker, caused by *Fusarium circinatum*, is a serious disease of *Pinus radiata* and also infects other pine species as well as Douglas fir. As the New Zealand plantation forest industry is heavily dependent upon *P. radiata*, this disease has the potential to cause serious losses if the fungus were to become established. The ability to rapidly detect and identify *F. circinatum* is a crucial component in maintaining the pitch canker free status of New Zealand; therefore, a DNA based diagnostic assay has been developed. The assay was developed based on bands that were consistently amplified across a sample population of *F. circinatum* following RAPD analysis. The conserved bands were sequenced and full-length PCR primers were developed that amplified only the target bands. The assay utilized 4 PCR primers in a multiplex PCR reaction that targeted two different regions of the *F. circinatum* genome. In order to assess the specificity of the assay, a total of 54 isolates of *F. circinatum*, representing a global population of the pathogen, and 34 species of other Fusaria, represented by 127 isolates, were screened through the assay. Statistical analysis of the results indicated that the assay had a 93.6 percent chance of identifying *F. circinatum* correctly and a 6.4 percent chance of giving a false negative. All 3 of the 54 isolates of *F. circinatum* that were not detected by the assay were members of VCG group 7. When the sample tested was not *F. circinatum*, it was correctly identified as negative with a reliability of 98.8 percent and the chance of a false positive reaction was 1.2 percent. One isolate of *Fusarium begoniae*, a close relative of *F. circinatum*, resulted in a positive reaction. The assay is able to detect *F. circinatum* in culture, in infected host plant tissue and in infested soil and potting mix.

S2.16p

PHYLOGENY OF SOUTH AFRICAN *RAVENELIA* SPECIES

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The rust fungi are recognized as including some of the most important tree pathogens. *Ravenelia* is the third largest rust genus with about 200 species recognized worldwide. These fungi mainly occur in tropical and sub-tropical regions of America, Asia, and Africa and are restricted to legumes (*Fabaceae s.l.*) and they predominantly infect tree species. Of the 24 *Ravenelia* spp. previously recorded from South Africa, based on morphology, 12 are found on various species of *Acacia*. The aim of this investigation was to understand the phylogenetic relationships of the South African *Ravenelia* spp. To achieve this goal, rusts were collected between January and June 2005 to obtain all relevant spore stages. These were examined using light microscopy to determine the morphological features. DNA was extracted from both aeciospores and teliospores and sequence data from the LSU, SSU, ITS, ß-tubulin gene regions were obtained from these collections. A preliminary phylogenetic analysis has enabled us to link the different stages of the life cycles of these rusts and to determine which morphological features are phylogenetically significant. As a result of this study we have identified various new hosts and one *Ravenelia* spp. new to South Africa.

S2.17p

EFFECTS OF TWO ECTOMYCORRHIZAL FUNGI ON THE ROOTING OF HYBRID ASPEN (*P. TREMULA* X *TREMULOIDES*) EXPRESSING HETEROLOGOUS HAEMOGLOBIN GENE *VHB*

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Mycorrhizal symbiosis refers to the mutualistic association between plants and certain soil fungi. This interaction offers several benefits to the host plants, including improved nutrition, drought resistance and protection against pathogens. The molecular research concerning mycorrhizal symbiosis is still at the early stage but it has been shown that expressions of certain endogenic haemoglobin genes of plants are regulated during arbuscular mycorrhiza (AM) formation. *Populus tremula* and *P. tremuloides* are the few species living in symbiosis with both AM and ectomycorrhizal (ECM) fungi. The aim of our work was to study effects of ECM fungi on the rooting of wild type and transgenic hybrid aspen (*P. tremula* x *tremuloides*) lines carrying *Vitreoscilla* haemoglobin gene (*vhb*). *In vitro* shoots of two non-transgenic and two transgenic lines of hybrid aspen were inoculated with the mycelium of *Leccinum populinum* isolated from pure hybrid aspen stand and *Xerocomus subtomentus* originally isolated from mixed aspen stand. The number of roots and mycorrhizas were measured after 3 weeks of culturing. The concentrations of jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) in shoot samples were analyzed by gas cromatography mass spectrometry and expressions of endogenic and heterologous haemoglobin genes are now under examination using Real Time PCR. Both fungal strains enhanced adventitious as well as lateral root formation, resulting in increased root fresh weight. However, only *L. populinum* formed mycorrhizas. No significant difference in the number of mycorrhizas was found between non-transgenic and transgenic hybrid aspens. Depending on the hybrid aspen line, the fungi decreased ABA concentration of the seedlings. Concentrations of JA and SA were unaffected by fungal inoculations.

S2.18p

A GENETIC LINKAGE STUDY OF AN INTERSPECIFIC CROSS BETWEEN *FUSARIUM CIRCINATUM* AND *FUSARIUM SUBGLUTINANS*

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Fusarium isolates associated with the *Gibberella fujikuroi* complex comprise many important fungal pathogens of agricultural crops and trees. Included are *F. circinatum* (the pitch canker fungus) and *F. subglutinans* that are pathogenic on susceptible pine sp. and maize, respectively. The complex currently consists of nine different biological species (mating population A-I) that are reproductively isolated. An interspecific hybrid cross in this complex between *F. circinatum* (mating population H) and *F. subglutinans* (mating population E) is providing a means of studying these two species using genetic linkage mapping. A framework linkage map was constructed using 582 amplified fragment length polymorphism (AFLP) markers together with the mating type (*MAT-1* and *MAT-2*) genes and a histone (H3) gene. Twelve major linkage groups were identified (n=12). An F_2 backcross population was then generated by backcrossing an F_1 hybrid isolate to the pitch canker parent. This population is being used to identify quantitative trait loci (QTLs) linked to pathogenicity in the pitch canker genome. Results of inoculations of 94 randomly selected backcross individuals on susceptible *Pinus patula* seedlings showed that lesion length had a normal distribution with a heritability (H^2) of 0.30. Although not high, this indicates an appreciable amount of genetic control for pathogenicity. AFLP analysis was performed on these backcross individuals using the same AFLP primer combinations previously used for genetic map construction in the F_1 cross. Identification of fungal pathogenicity QTLs will provide powerful tools to study the genetic architecture of interspecific differentiation of pathogenicity in *Fusarium*.

S2.19p

CYLINDROCLADIUM PAUCIRAMOSUM: A THREAT TO *EUCALYPTUS* CUTTING PRODUCTION IN SOUTH AFRICA

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In South African *Eucalyptus* nurseries, damage to plants by *Cylindrocladium* is common and reflected by a number of disease symptoms. *Cylindrocladium* was first recorded on *Eucalyptus* in South African nurseries in 1988 where it caused damage to hybrid cuttings. Subsequently, several *Cylindrocladium* spp. have been recorded from local *Eucalyptus* nurseries. A survey was conducted at four major *Eucalyptus* cutting production nurseries in KwaZulu Natal, South Africa, to determine which *Cylindrocladium* is dominant. At each nursery, five of the most commonly produced hybrid clones were selected for sampling. Cuttings were immediately examined and placed in moist chambers to enhance fungal sporulation. Several well-known fungal pathogens were collected and amongst these, *Cylindrocladium* isolates were dominant. All *Cylindrocladium* isolates were identified as *C. pauciramosum* based on morphological characteristics and this was confirmed using DNA sequence data comparisons based on part of the *f*Ò-tubulin gene. The pathogenicity of *C. pauciramosum* was tested on two-month-old plants representing four commercial hybrid clones. The pathogen gave rise to infection on all clones inoculated with little difference in tolerance noted between these clones.

S2.20p

REAL-TIME RELATION BETWEEN PATHOGEN GROWTH AND DEFENSE GENE EXPRESSION, THE REALITY SHOW OF PLANT-PATHOGEN INTERACTIONS

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Obligate biotrophic pathogenic fungi cause important problems in agriculture and forestry. Their study is particularly challenging because of our inability to obtain axenic cultures. To circumvent this problem and to study interactions between hybrid poplars and poplar rust fungi *Melampsora medusae* and *M. larici-populina*, we have developed a quantitative real-time PCR based methodology to monitor the in-planta growth of fungi. This methodology provides unprecedented sensitivity for monitoring fungal growth, and the specificity required to distinguish pathogens in mixed infections. Moreover, real-time PCR has been widely used to monitor gene expression. Here we propose a combined approach where defence gene expression is related to pathogen growth in time course experiments. Monitoring of gene expression following *Melampsora* inoculations on poplar clones harbouring various levels of resistance has revealed numerous interesting facts. 1) Our results confirmed that compatible interactions weakly induce the host defence gene expression even when a high amount of pathogen is detected. 2) New insights on the timing of defence gene induction in relation to pathogen amount were revealed by the study of various types of resistance reaction. 3) A mixed inoculation experiment with virulent and avirulent pathogens has given a surprising outcome for both pathogen growth and defence gene expression.

S2.21p

INNATE DEFENSE IN CONIFER OVULES RECONSIDERED: PROTEOMICS POINTS TO POLARIZED DEFENSES IN GYMNOSPERMS?

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The gymnosperm ovule is complex in its construction. At reproductive maturity, its center is occupied by a large haploid egg-containing megagametophyte that is surrounded by diploid nucellar tissue and an integument. The apex of the ovule is formed into a micropyle, the point of entry of the pollen. The innate defence barriers differ according to location. A waxy cuticle protects the outer epidermis of the integument. The micropyle is defended by a waxy layer on the inside of the integument as well as by ovular secretions that are rich in antimicrobial compounds. These include a variety of small and large molecular weight compounds, ranging from organic acids to defence proteins. These antibacterial and antifungal compounds are secreted by the nucellus. The megagametophyte is protected over most of its surface by a membrane. The megagametophyte, unlike the sporophyte's nucellus, does not appear to have a suite of defence compounds at the ready, as is implied by a unique type of insect parasitism that occurs in the Pinaceae. Pathogen or pest interaction with reproductive tissues of the ovule is strongly differentiated by location. Examples include Douglas fir, larch, yew, Port Orford cedar, yew and *Welwitschia*.

S2.22p

STREPTOMYCETES FROM THE RHIZOSPHERE OF SPRUCE DIFFERENTIALLY AFFECT SYMBIOTIC AND PATHOGENIC SOIL FUNGI

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The rhizosphere, the narrow zone of soil around living roots, is characterized by root exudates which attract soil microorganisms. Most importantly, certain soil fungi establish symbiotic interactions with fine roots, which enhance nutrient availability for the plant partner (mycorrhiza). The establishment of such a symbiosis can be affected by soil bacteria. We isolated Gram-positive soil bacteria from the rhizosphere of a spruce stand rich with fly agaric (*Amanita muscaria*) fruiting bodies. Using a coculture technique in Petri dishes, bacterial isolates were characterized by their effect on the growth of fungal hyphae. A group of bacterial strains were identified which significantly promoted growth of fly agaric hyphae. One of these strains (*Streptomyces nov.* sp. 505 (AcH 505)) was shown to additionally inhibit growth of pathogenic fungi such as *Armillaria obscura* (wide host range) and *Heterobasidion annosum* (causes wood decay in conifers). In coculture with fly agaric (*Amanita muscaria*) we found strongest promotion of growth of the fungal mycelia after 9 weeks. In addition to morphological changes we examined the effect of AcH 505 on fungal gene expression using the suppressive subtractive hybridisation approach. The responsive fungal genes include members of signalling pathways, metabolism, cell structure, and cell growth-response. Furthermore we were able to isolate and characterize their bacterial substances, released to the medium, which induce the same effects as the bacteria. One of them has not yet been described.

S2.23p

GENOTYPIC DIVERSITY OF THE ROOT ROT FUNGUS *ARMILLARIA FUSCIPES* IN SOUTH AFRICAN PINE PLANTATIONS

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Armillaria fuscipes is the causal agent of *Armillaria* root rot in pine plantations in Southern Africa. The taxonomy of this fungus has been well established but its population structure in pine plantations is unknown. The aim of this study was to ascertain the genetic diversity of *A. fuscipes* in *Pinus elliottii* and *P. patula* plantations in South Africa. Isolates were collected from plantations in the Mpumalanga and Limpopo provinces. RFLP analyses of the IGS-1 region were performed on the fungal isolates to confirm their identity. The genotypes of the isolates were assessed using vegetative incompatibility. These tests were conducted by crossing all the isolates with one another in all possible combinations on malt extract agar. Genetically identical isolates were identified by the absence of a demarcation line between two crossed isolates. AFLP analyses were employed to further assess the genetic diversity of the isolates. Banding patterns for these analyses were obtained from three primer sets. RFLP profiles typical of *A. fuscipes* were obtained for all isolates. Thus, only this species is present in pine plantations in South Africa. Isolates collected within discrete infection centers were found to represent single clones of *A. fuscipes*. Preliminary results on the isolates from different centers suggest that they each represent different genotypes. Assessment of the total number of clones and their genetic relationships to one another will provide information on the genetic history of *A. fuscipes* in South Africa and assist in the management of this pathogen in pine plantations.

S2.24p

LEPTOGRAPHIUM SPP. ASSOCIATED WITH BARK BEETLES INFESTING CONIFERS IN CHINA

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Leptographium spp. are often associated with bark beetles. They are also known as sapstain agents of lumber and conifer root pathogens. Very little research has been done on this group of fungi in China. In the past three years, we carried out a survey of the Ophiostomatoid fungi associated with conifer-infesting bark beetles in China, and obtained a large fungal collection. Preliminary identification based on morphology indicated that 18,5 % of the isolates collected belong to the genus of *Leptographium*. The aim of this study was to identify these isolates. The isolates were grouped according to the morphological characteristics, and confirmed by comparisons of DNA sequences of the partial â-tubulin gene. Isolates were allocated to twelve morphological groups and the phylogenetic analysis showed they represented the species of *L. terebrantis* Barras & Perry, *L. yunnanense* Zhou, Jacobs, Wingf. & Morelet, *L. truncatum* (Wingf. & Marasas) Wingf., *L. bistata* Kim, Lim, Wingf., Breuil & Kim and *Ophiostoma penicillatum* (Grosmann) Siemaszko, are first reported from China. Some isolates however did not match with known *Leptographium* spp., and probably are undescribed taxa. Sequences of other genes will be used to confirm the identities of those isolates.

S2.25p

MOLECULAR CHARACTERIZATION OF THE INTERACTION BETWEEN SIRICID WASPS, THEIR FUNGAL SYMBIONTS AND TREE HOSTS

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Some siricid woodwasps (Siricinae, Hymenoptera), such as *Sirex noctilio*, have been introduced into non-native areas where they cause significant damage to local softwoods. These woodwasps live in an obligate, mutualistic symbiosis with fungi in the genus *Amylostereum* (Basidiomycotina). Female woodwasps inoculate softwood trees with the fungus together with phytotoxic mucus, when they lay their eggs. The fungus then causes a white-rot that is essential for the development of wasp larvae. Siricid woodwasp species are always associated with the same *Amylostereum* sp. (either *A. areolatum, A. chailletii* or *A. laevigatum*) and often also display a certain level of host tree preference. The European wasps, *Sirex juvencus* and *S. noctilio* both live in associations with *A. areolatum*, but *S. juvencus* preferentially feeds in *Picea*, while *S. noctilio* prefers *Pinus*. Here we studied the phylogenetic and population structure of different woodwasps and their *Amylostereum* symbionts using DNA based molecular markers. This gives us the opportunity to determine the extent to which genetic differentiation of symbionts might determine host tree preference of the wasps. These data are used to consider the potential for future spread of different wasp species, as well as its significance in breeding for resistance to the symbionts of these organisms.

S2.26p

APPLICATION OF A PATHOGEN-INDUCIBLE NATIVE TREE PROMOTER TO ENGINEER DISEASE RESISTANCE IN TRANSGENIC POPLAR

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Despite increasing commercial value, few *Populus* species combine desirable growth characteristics with sufficient resistance against major tree pathogens. Expression of defensive genes encoding membrane-active cationic antimicrobial peptides, especially when controlled by a promoter with predictable spatiotemporal activity, provides a unique opportunity to engineer plant resistance against a wide array of pathogenic microorganisms. Previously we showed that wound-inducible *win3.12T* promoter from hybrid poplar (*Populus trichocarpa x P. deltoides*) had strong systemic activity in the aerial parts of plants in response to pathogen infection, whereas in the absence of stimuli its transcriptional activity was hardly detectable. Based on these findings, a plant transformation vector with transcriptional fusion between the *win3.12T* promoter and the *MsrA2* gene, which encodes a small, dermaseptin B-based cationic peptide with strong antimicrobial properties, was constructed and introduced into hybrid poplar (*P. nigra x P. maximowiczii*) via *Agrobacterium*-mediated transformation. As a proof of concept, this DNA construct was also introduced into tobacco. Development of a new regeneration protocol and explant-dependent selection conditions dramatically improved transformation efficiency and increased the number of positive transgenic poplar lines. Stable transgene integration into plants regenerated on selective medium was confirmed by PCR and Southern analyses, whereas northern analysis showed accumulation of *MsrA2* transcripts in response to fungal infection. All transgenic plants had the normal phenotype, with no indication of cytotoxicity due to expression of the *MsrA2* gene. Cultivation of transgenic plants in the presence of *Fusarium solani* showed that the pathogen-induced accumulation of the MsrA2 peptide was sufficient to confer plant resistance to the pathogenic fungus. Bioassays of transgenic poplar against several major pathogens are in progress.

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S2.27p

TRANSGENIC POPLARS ALTER INTERACTIONS WITH NON-TARGET PATHOGENS

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There is a major concern that the release of genetically modified trees (GM-trees) will result in unexpected effects on non-target organisms, but there are virtually no data available to assess these risks. In order to identify and assess potential non-target effects of the field release of transgenic trees we used a model system consisting of the transgenic poplars *Populus tremula* x *tremuloides*, line T89, featuring changes in growth characteristics, i.e. altered expression of gibberellin (GA 20-oxidase), sucrose-phosphate synthase (SPS) and pectin methyl esterases (PME), and five and six populations of the non-target parasitic fungi *Venturia tremulae* and *Melampsora pinitorqua*, respectively. In inoculation experiments (cut-shoot and leaf-disc assays respectively) we compared the different transgenic lines susceptibility to the pathogens with the base-line, represented by the unmodified isogenic line T89. We found that all tested transgenic lines altered the susceptibility compared to the unmodified wild type T89 for both of the pathogens, but also that the susceptibility varied considerably within lines depending on expression level of the transformation, and depending on pathogen population. This is one of the first experimental studies that have shown that genetic modification of a tree is capable of altering the interaction with a non-target pathogen. The results are principally important because much attention in forest biotechnology today are directed to develop trees with non-target traits, to be used in forestry applications, and natural enemies such as pathogens are important actors in forest ecosystems, capable to regulate host populations and drive ecological and evolutionary processes. The results highlight the importance and necessity of the implementation of risk assessments before field-release and commercial application of transgenic trees.

S3. Breakthrough and high-throughput technologies for functional and structural genomics in trees

S3.1

METABOLITE PROFILING OF *POPULUS*: INSTRUMENTATION, CHEMOMETRICS AND DATABASES FOR INTEGRATION IN TREE BIOTECHNOLOGY

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Metabolomics has the last years emerged as an important tool in very many different aspects of biology. We have developed a metabolite profiling approach including both GC/MS and LC/MS analysis, automated MS-data processing concepts, multivariate statistical analysis, and databases. We will present the whole concept, but the focus will be on showing the importance to use different analytical platforms and analyse the large and complex datasets with methods such as Principal Component Analysis (PCA) and Partial Least Squares (PLS). The latter not only for visualization purposes but also for validation of data and finding the metabolites that explains the differences between samples. We will show example from different *Populus* project where the metabolomics concept has been used, e.g. induction of dormancy in *Populus*.

S3.2

A FUNCTIONAL GENOMICS APPROACH TO IDENTIFYING POTENTIAL REGULATORS OF TREE GROWTH, DEVELOPMENT AND DEFENSE RESPONSES

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Forest tree growth, development and defence responses are exquisitely dynamic processes that are modulated by a variety of genetic and environmental cues. We are using a functional genomics approach to characterize regulators that influence these processes in two forest tree taxa, poplar and white spruce. To this end, we have selected as candidates for functional analyses a number of genes from forest trees that we hypothesize are regulators of wood formation and potential players in the defence response. As a means to further define the role that these genes play, a suite of transgenic poplar and spruce trees has been created that miss-express each of the candidate genes. Large-scale transcript profiling with custom microarrays is being used to assess how these candidate genes and environmental conditions act to modulate gene expression.

FOTO

S3.3

THE NEED FOR HIGH-THROUGHPUT BIOCHEMICAL GENOMICS: TERPENOID SYNTHASES AND P450 ENZYMES IN CONIFER DEFENSE

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Cytochrome P450 monooxygenases (P450s) are important enzymes for generating some of the enormous structural diversity of plant terpenoid secondary metabolites. In conifers, P450s are involved in the formation of a suite of diterpene resin acids (DRAs). Despite their important role in constitutive and induced oleoresin defense, a P450 gene of DRA formation has not yet been identified. Using phylogenetic cluster analysis of P450-like expressed sequence tags from loblolly pine (*Pinus taeda*), functional cDNA screening in yeast (*Saccharomyces cerevisiae*), and in vitro enzyme characterization, we cloned and identified a multi-functional and multi-substrate cytochrome P450 enzyme, CYP720B1, abietadienol/abietadienal oxidase (PtAO). PtAO catalyzes an array of consecutive oxidation steps with several different diterpenol and diterpenal intermediates in loblolly pine DRA biosynthesis. Recombinant PtAO oxidized the respective carbon-18 of abietadienol, abietadienal, levopimaradienol, isopimara-7,15-dienol, isopimara-7,15-dienal, dehydroabietadienol, and dehydroabietadienal with apparent Michaelis Menten (Km) values of 0.5 to 5.3 mM. PtAO expressed in yeast also catalyzed in vivo oxidation of abietadiene to abietic acid, but with activity much lower than with abietadienol or abietadienal. Consistent with a role of DRAs in conifer defense, PtAO transcript levels increased upon simulated insect attack using methyl jasmonate treatment of loblolly pine. The multi-substrate, multi-functional P450 diterpene oxidase PtAO, in concert with expression of a family of single- and multi-product diterpene synthases, allows for formation of a diverse suite of DRA defense metabolites in long-lived conifers.

Ro D-K, Arimura G-I, Lau SYW, Piers E, and J Bohlmann (2005) Loblolly pine abietadienol/abietadienal oxidase PtAO is a multifunctional, multi-substrate cytochrome P450 monooxygenase. Proceedings of the National Academy of Sciences USA 102: 8060-8065

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S3.4

FUNCTIONAL GENE TESTING IN *PINUS RADIATA* CALLUS CULTURES

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Functional testing of cell wall related genes in conifers such as *Pinus radiata* can be a time consuming and labour intensive process, if these experiments depend on the production of transgenic trees. Even small plants, which can be used for cell wall related studies, generally require more than one year of development. We have therefore developed a xylem derived *P. radiata* callus culture system, which is both transformable and capable of forming tracheary elements. The produced secondary cell walls seem to have a similar chemical fingerprint compared to those formed in developing xylem. This callus culture system has several advantages over plant forming material, since the generation of transgenic lines is both easy and fast. In addition, physiological manipulations and gene expression studies can easily be undertaken. Data will be presented on recent experiments with transgenic callus cultures providing evidence that the system can be used for functional testing of cell wall related genes. The expression levels of the lignin related genes were manipulated in a pilot study using RNAi technologies. Initial results of the impact of this gene silencing approach on metabolite profiles in transgenic lines will be presented. In summary, we believe that our recent results indicate that the established *P. radiata* callus culture system is useful as a screening tool to investigate the function of cell wall related genes and is also suitable as a model for metabolic engineering experiments.

fotos

S3.5

COMBINING METABOLOMICS AND QTL ANALYSIS FOR IDENTIFYING MQTL AND GENE DISCOVERY IN POPLAR

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Metabolomics or metabolic profiling offers tremendous potential to discover novel genes, and assign function to those genes. Gene discovery by metabolic profiling is a rapidly emerging field that will employ multiple approaches, including the coupling of metabolic profiling with the analysis of 1) mutant and transgenic lines, and 2) structured pedigrees to identify metabolite quantitative trait loci (mQTL). Poplar (*Populus*) mutants containing a single-gene modification can express greatly perturbed metabolism, leading to the overproduction or depletion of metabolites of both primary and secondary carbon and nitrogen metabolism. Such single-gene mutations typically induce changes in metabolic profiles that are highly pleiotropic and often difficult to predict. When the metabolite data of the fine roots of the progeny of an interspecific backcross between *P. trichocarpa* X *P. deltoides* '52-225' and *P. deltoides* 'D124' (Family 13) at Thief River Falls, MN, was subjected to QTL analysis, a number of mQTL were identified, including a large-effect mQTL that explained 16.9% and 26.8% of the progeny variation in the concentration of trichocarpinene and its glucoside, trichocarpin, respectively. In summary, extensive biochemical characterization of unique metabolic phenotypes of both mutants and segregating progeny (backcross and F2) can provide insight into gene function and how metabolic pathways are interconnected. In addition, elucidating these biochemical networks will provide opportunities for tailoring metabolic engineering for the overproduction of unique secondary carbon metabolites.

FOTO

S3.6

CHARACTERIZATION OF REGULATORY GENES IN THE SECONDARY MERISTEM OF *POPULUS* BY IN SITU PROTEIN LOCALIZATION

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Trees maintain their stem cells population for hundreds of years, with continuous production of biomass as a result. However, the genetic mechanisms for regulation of growth in secondary meristems are essentially unknown. We aim to find and characterize the genes involved in this process in *Populus* in order to understand if increased productivity can be reached by manipulation of the pool of stem cells, the xylem and phloem mother cells or the differentiating cells. The goals will be achieved by functional analysis of potential target genes. Currently, the tools for this type of analysis include RNAi knock-down and overexpression under cambium-specific promoters. This study aims to introduce a complementary approach based on *in situ* protein localization, which allows for higher resolution and analysis of protein activity on the cellular and sub-cellular level. Polyclonal antibodies to the target proteins will be produced in using a sophisticated antigen design procedure, which allows for selection of unique epitopes. The specificity to the target proteins is increased by affinity purification of the antibodies using the antigen. Tissue sections of the meristem will be stained by immunohistochemical methods. To date, almost 80 candidate genes from the cambium have been identified through microarray analysis, 40 of which are in the process of antibody generation. More than 200 transgenic poplar trees are available from a commercial partner through collaboration with SweTree Technologies (www.swetree.com).

S3.7

THE INTRICACIES AND INCENTIVES OF HIGH-THROUGHPUT PROTEOMICS IN ITS APPLICATION TO TREE BIOLOGY

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A thorough investigation of any dynamic biological system requires multiple approaches. The development of high-throughput sequencing and microarray technologies has made genomics a dominant form of research. In spite of all the advantages associated with these techniques, the additional information that can be obtained through proteomics can greatly improve the depth of biological insight gained. Proteomics, along with metabolite profiling and bioinformatics, has provided a versatile and complementary approach to high-throughput biological investigations. Protein abundance, post-translational modifications, protein turnover, and noncovalent interactions are all significant components of any biological system. Proteomics encompasses the study of all these aspects at the price of high technical complexity. With the development of soft ionization techniques, mass spectrometry has become an integral component of any protein scientist's research arsenal. Isotopic labeling (ICAT and iTRAQ) has provided a means for large-scale quantitative experiments that can accurately measure thousands of proteins from a single biological sample in a matter of hours. In addition, differential scanning techniques can identify post-translational modifications with known structures or chemical properties. However, the application of these technologies to tree biology is hindered by the challenges inherent in processing the relevant biological materials. It must be further appreciated that, with respect to conifer species, no direct genomic resource is available to assist in data analysis. In spite of these difficulties, the information arising from carefully applied studies in proteomics have exponentially aided our understanding of tree biology in terms of both defense and basic development. In combination, with transcript profiles and metabolite studies, we have gained a more thorough understanding of the processes that directly affect the health and welfare of our forests. We have applied the technologies associated with proteomics not only to model organisms like *Arabidopsis* and poplar, but also to coniferous species such as spruce. The intricacies of the methodology ranging from 2D-PAGE to isotopic labeling to multiple reaction monitoring applied in our studies have provided us with relevant biological information, which merits the use of disparate methods of analyses in order to entirely comprehend any system associated with tree biology.

S3.8

A BAC LIBRARY OF *EUCALYPTUS GRANDIS*: CHARACTERIZATION, FINGERPRINTING, BAC-END SEQUENCING AND SHOTGUN ASSEMBLY OF LIGNIFICATION GENES

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A *Eucalyptus grandis* BAC library (HindIII partial digest) with an initial coverage of ~4X with over 70% of the inserts >150 kb long, has been built in the Genolyptus project. A hierarchical pooling strategy was employed to rapidly identify BAC clones containing a gene or marker of interest. BAC DNA pools were done by pooling the 96 clones from a single plate. The 210 DNA pooled samples were in turn pooled in 21x10 or 35x6 superpools x pools (96-well plates). Step one of the BAC library screening was carried out with PCR reactions on the superpools plus the genomic DNA control. Pools comprised in a positive superpool were then screened and the 96-well plate corresponding to the positive pool was screened by colony-PCR to arrive to the target BAC, thus totalling 21+10+96= 127 PCRs to get to a gene or 137 for the 35 superpool format. This library was validated by screening it with 47 mapped microsatellites that pulled out on average 3.7 BAC clones thus confirming the expected coverage. These anchored BACs are being used to integrate the molecular and cytogenetic maps via fluorescence in situ hybrization (FISH) techniques). Using this BAC library we have also undertaken a directed search for the genomic clones of a number of candidate genes involved in wood chemical composition and disease resistance for subsequent shotgun sequencing. Primers were obtained both from the literature as well as from consensus sequences in ORFs derived from *Eucalyptus* EST clusters. This strategy has allowed us to land on BACs for important genes that code for enzymes and transcription factors involved in the lignin or cellulose biosynthetic pathway as well as for resistance gene analogs (RGAs). The same screening strategy was used to identify groups of 4 or more BAC clones pulled out by screening the library with the same single locus microsatellite marker heterozygous in the donor tree so that both alleles were likely represented in the group of BACs. BACs within each group are now being fingerprinted and assembled into contigs to evaluate the robustness of the fluorescent fingerprinting approach. A sequence-tagged connector (STC) resource has also been created by sequencing both BAC ends of the first arrayed set of 20,160 clones. This has been accomplished with an average efficiency of 78% at phred> 20 on 250 bp. The ~31,000 STCs should, on average, be scattered every 20 kb across an estimated 630 Mbp genome. Once any BAC is sequenced to completion by a shotgun approach, these STC tags can be used to identify a minimum tiling path of BAC clones overlapping the nucleation sequence for sequence extension. At this STC density, any nucleation BAC (insert size of 150 kb) should have around 7 STCs. The STCs may hit mapped markers (e.g., STSs, ESTs, microsatellites etc.) and, thus, map the corresponding BAC clones to precise chromosomal locations or, on a reverse approach the STC provide rich sources of new microsatellites or SNPs for the same purpose. In fact over 1,800 new microsatellites were already mined from

the STC database. For two key genes, 4-CL and CAD, the smaller single BAC (30Kbp for CAD and 120 kbp for 4-CL) as evaluated by PFGE was selected for constructing shotgun libraries with average insert size of 2 kbp of which both ends were sequenced. Assembly of 1,052 reads (3.5X) for 4-CL resulted in a 5,477 bp contig covering the whole gene but part of the 5-UTR. For CAD 768 reads (10X) allowed the assembly of a 9,785 bp contig of what was found to be CAD2. With the full genomic sequence in hands it should be possible to identify regulatory regions and carry out a detailed analysis of polymorphism in a set of individuals by resequencing specific upstream regions in an association mapping approach.

S3.9p

A COMPARISON OF DIRECT (FLOW CYTOMETRY) AND INDIRECT (STOMATAL GUARD CELL LENGTHS AND CHLOROPLAST NUMBERS) TECHNIQUES AS A MEASURE OF PLOIDY IN BLACK WATTLE, *ACACIA MEARNSII* (DE WILD)

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Three assays, namely stomatal length measurements, chloroplast numbers within the stomatal guard cells and flow cytometry, were used to confirm the ploidy of three diploid and three tetraploid *Acacia mearnsii* lines. The first two methods have previously been successful in identifying ploidy in this species, but they are indirect assessments. Flow cytometry directly quantifies the amount of DNA present in a sample, but has not been previously tested on *A. mearnsii*. It was decided to test the accuracy of all three methods by using the exact same plant material for each method. Results showed that each method correctly identified the ploidy of the diploid plant material tested as well as two of the three tetraploids tested. The third tetraploid (C19/48/17) was originally identified as a tetraploid, however all three techniques identified it as a diploid, suggesting that it was originally incorrectly identified as a tetraploid. From this study, all three techniques followed the same trend, confirming their accuracy for future research into ploidy identification of *A. mearnsii*. Flow cytometry has the advantage of providing a quick and efficient direct assessment of DNA in the sample, but is the most expensive. The other two techniques used (stomatal length measurements and chloroplast numbers within the stomatal guard cells) are also accurate but are indirect methods and more time consuming.

S3.10p

DEFENSE RESPONSE IN POPLAR: A COMBINED APPROACH OF TRANSCRIPT PROFILING AND FUNCTIONAL ANALYSIS

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Transcriptional regulators play strategic roles in plant development and in response to the environment. Plant defence response to microbial attack is regulated through a complex network of signalling pathways involving different signalling molecules such as jasmonic acid (JA) and salicylic acid (SA). These signalling events eventually lead to the activation of different transcription factors that control defence genes expression. In this project we focused on plant defence candidate genes, mainly transcriptional regulators (e.g. EREBP, MYB, TGA, and WRKY), but also others involved in various aspects of the plant defence response (e.g. NPR1). In a first step, some of these genes were characterized and have been shown to be induced following wounding, JA or SA treatments. In a second step, functional analyses of these candidate genes were undertaken by ectopic expression in poplar. Transgenic poplars were challenged with Melamspora and pathogen growth was measured by quantitative RT-PCR. Some of the transgenic lines showed enhanced susceptibility to poplar rust whereas others demonstrated enhanced resistance. The next challenge will be to highlight the involvement of these candidate genes in signal transduction steps and defence response resulting from the tree-rust interaction.

S3.11p

TRANSFORMATION SYSTEMS FOR THE CREATION OF SOMATIC WOOD SECTORS IN TREES CAN AID THE FUNCTIONAL ANALYSIS OF GENES INVOLVED IN XYLOGENESIS

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The functional analysis of genes involved in xylogenesis is hindered by obstacles such as long generation times, biochemical characteristics of woody tissue and low transformation efficiencies. Many of these obstacles may be avoided by combining *in vitro* and *in vivo* wood formation systems with the transfer of foreign genes directly to target cells within the growing stem through *Agrobacterium*-mediated transformation. The usefulness of such approaches for studying specific gene effects within somatic sectors, which clonally derived from transformed target cells, and their application for the study of pattern formation during cambial development and differentiation is now being demonstrated. This poster describes and summarises the range of new methods developed in our laboratory, which dramatically reduce the time needed from initial transformation to the analysis of (trans-) gene effects in xylogenic tissue from several years to only a few months. These methods include transformation systems for *in vitro* stem cultures, *in vivo* stem inoculation and dormant bud transformation in a variety of commercially important tree species including eucalypts, poplar, acacia and pines.

S3.12p

A TIMETABLE OF ASPEN LEAF DEVELOPMENT

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Many trees have an indeterminate growth, forming leaves throughout the growing season and leaf plastochron index (LPI) can be used to describe the different leaf developmental stages down the stem. Fully grown aspens and many other tree species in northern Europe, only have one flush so at a given date, all leaves are of identical age, making it a good system to study leaf development. Here a genomic and multivariate statistic approach was chosen to analyse gene regulation of aspen leaves in their natural environment. Microarrays were used to measure mRNA levels in leaves collected from a free-growing aspen tree (*Populus tremula*) during the whole growing season (which in Umeå is about end of May to October). First, samples from eleven days, similar according to measured weather parameters and evenly spread over the growing season, were selected to pinpoint developmental changes and disregard weather-dependent changes. We have been able to separate the season into three main phases. During the first month in the spring, developmental factors were the main determinants of gene expression. In the autumn, senescence regulates gene expression, but in the middle-phase, gene expression was mainly dependent on environmental factors. Secondly, the focus was on the developmental phase and thirteen samples from two years were analysed by microarrays. The leaf transcriptome underwent huge changes during the first weeks of leaf development and during this stage, a predefined developmental program of the leaves were much more important than environmental parameters in determining the leaf transcriptome. The major impact of weather during this stage was that the ambient temperature determined the speed of progression through the different developmental stages, and we postulate that the temperature sum is the major determinant for the rate of development of aspen, and potentially all tree, leaves. This phase were divided into four developmental stages corresponding to cell division, cell elongation, primary cell wall biosynthesis through a transition stage to the secondary cell wall metabolism. Together with experiments analysing other factors (e g abiotic and biotic interaction, nutrient factors and circadian rhythms), our aim is to cluster all expression data and define the different regulons in Populus leaves. Single gene technologies (e.g. Real-Time RT-PCR) will be applied on the regulons to get large-scale information about gene regulation in a high-throughput manner.

S3.13p

A MULTI-SPECIES COLLECTION OF PLANT ESTS: AN OPEN SOURCE FOR MICROARRAYS

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After the genomics area, functional and comparative genome analysis will be the most interesting field of research for the upcoming years. With the emergence of huge data sets on ESTs (expressed sequence tags) on various plant species, we are facing the problem on how to work with these decentralised resources. To address the full potential of functional diversity globally available in plants, the understanding of interspecific related-ness of gene functions is a timely and highly important objective and will play a key role in future plant improvement programs. Especially in functional genomics, the prompt availability, management and standardisation of resources and data is a key factor for successful research. In order to strengthen this research segment, we are setting up a resource centre for plant ESTs (www.picme.at) providing the plant science community with DNA-microarrays carrying ESTs from a variety of species for various research purposes (oak, pine, poplar). These ESTs are derived from different academic institutions and consortia cooperating in an international network, thus providing resources from a single source. This allows researchers concentrate on their research of interest, not having to deal with clone management, maintenance or distribution protocols (1). In the frame of a European Network of Excellence the collections will be even expanded to provide reference DNA samples for population genetic analysis as well as functional characterizations. The importance of such a repository centre will be highlighted, discussing the benefits for boosting international research. Based on the premise that many genes in plants are shared with considerable functional conservation, there is an opportunity to exploit the EST collections in order to develop thematic micro arrays that might work on a wider range of closely related species. Based on these ideas, we are constructing cDNA arrays for investigating abiotic stress reactions of plants using high throughput expression profiling. Based on literature data for model species we select orthologous probes from the physical cDNA (EST) resources and print them on slides. This drought stress cDNA array will be tested on various related species. Data will give an idea on the potential transferability of ESTs across species.

S3.14p

LINKING PHYSICAL AND GENETIC INFORMATION TO UNDERSTAND LEAF DEVELOPMENT IN *POPULUS*

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Leaf size is an important determinant of biomass yield in *Populus*. Here we wish to understand the genomic and genetic control of leaf size and shape development by bringing together a number of morpho-physiological, genetic and genomic approaches. Firstly, in the F_2 Family 331 (POP1) cross between *Populus deltoides* and *P. trichocarpa* over five years and several experiments at contrasting sites and conditions, we have identified powerful QTL that contribute to leaf development. These have been used to define a list of candidate genes, now placed on the physical sequence and co-located to QTL. Complementary to this, we have analysed leaf development in a wide population of *P. nigra* selected from divergent latitudes across Europe and with contrasting leaf size and shape, now grown at a single site in Belgium and suitable for LD and association studies. Using this and the Family 331 resource we have analysed global patterns of gene expression using a *Populus* microarray from 'extreme' genotypes for leaf size and leaf shape. Altogether these give us a powerful insight into the likely genes of importance for leaf area development. SNPs detection is ongoing and the use of association mapping to some of these traits will be presented.

S3.15p

MICROARRAY ANALYSIS OF WITHIN-TREE AND BETWEEN-TREE VARIATION IN GENE EXPRESSION IN *EUCALYPTUS* WOOD-FORMING TISSUES

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Accurate analysis of gene expression patterns during wood formation in trees requires a thorough understanding of the underlying spatial variation in gene expression across the main stem of the tree, as well as, biological variation within the same tissues sampled from clonal replicates (ramets) of the same genotype, or from different genotypes (clones). We used a spotted cDNA microarray to analyse the expression profiles of 2608 genes in mature xylem, immature xylem and phloem tissues sampled from two heights (2 and 7 meters) on the main stem of a six year-old *Eucalyptus* hybrid tree. The same set of genes were also analysed in the immature and mature xylem tissues of three ramets each of two hybrid eucalypt clones. The data was analyzed using a mixed-model ANOVA approach in Statistical Analysis Software (SAS). A total of 1285 gene transcripts (49%) varied significantly ($P<0.00001$) in abundance within the stem of the six-year old tree (among tissues and stem positions). Ten major gene expression clusters (patterns) were identified across the three tissues and two stem heights. Using the three ramets of each clone as biological replicates, we were able to identify a smaller subset of 470 genes (18%) with transcripts that varied significantly between the two clonal genotypes (within immature and mature xylem tissues). A total of 498 gene transcripts (19%) exhibited variability in abundance ($R^2<0.7$) among clonal replicates (within clonal genotype). This information on intrinsic variation in gene expression in *Eucalyptus* trees is guiding us in performing more sophisticated comparisons, which will examine gene expression variation under various environmental conditions and developmental stages.

S4. Forest biotechnology adoption and the impact of the economic, scientific and societal value chains

S4.1

HOW BIOTECHNOLOGY WILL IMPACT THE FOREST PRODUCTS INDUSTRY: THE ECONOMIC VALUE CHAIN

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The value of biotechnology applications developed for use by the forest products industry is their *potential value* adjusted for critical uncertainties. Three significant uncertainties have to do with the probability of *technical success*, the probability of *commercial success*, and the probability that the *health, safety, legal, regulatory, and environmental* (i.e., *social*) aspects are successfully addressed so that the potential scale of implementation is realized. The risk-adjusted value of the technology is the potential value multiplied by these probabilities. There is very little doubt that the potential value of forest biotechnologies to the forest products industry is enormous. However, the size of the risk-adjusted value is much more uncertain. Two examples of biotechnology applications – cloning via somatic embryogenesis and creation of insect resistant pine, using genetic engineering to insert a BT-gene – are used to illustrate both the potential of the technologies and the magnitude of and actions required to address the uncertainties. These examples demonstrate the danger of too much focus on the potential value of biotechnology. For both of the technologies, all three sources of uncertainty are potentially significant and must be accounted for in any valuation analysis. The way in which the forest products industry addresses social uncertainties in both cases will have a profound impact on public perception and profitability.

FOTOS

S4.2

THE BENEFIT OF THE APPLICATIONS OF BIOTECHNOLOGY TO FORESTRY

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Over the next 50 years, human demand will put extreme pressure on our natural forests unless wood harvests can be shifted towards highly productive forest plantations. Biotechnology will be an important tool in the technology toolbox for sustaining the world"s forests. Improved genetics, provided through clonal forestry and biotechnology, together with improved silviculture and plantation management practices, will be required to meet the wood demands of the future. Genomic research is expanding the forestry industry"s capabilities to identify and utilize molecular techniques toward tree improvement. ArborGen, a forest biotechnology company dedicated to improving the sustainable productivity of plantation forests, is developing plantation forestry species with improved wood properties, growth and stress tolerance. Transgenic tree product development requires multiple competencies, and ArborGen has invested several years towards developing the following platforms: 1) elite genotypes as a genetic base for transgenic products, 2) elite clone transformation capabilities, 3) gene licensing or discovery for introduction of valuable traits, 4) tree performance assessment in the greenhouse and the field, 5) quality assurance and regulatory safety, 6) commercial level scale up of transgenic tree products, and 7) marketing and public acceptance. ArborGen has made significant progress and effort in all of these key areas, with an expectation that we will develop improved plantation forestry trees that can benefit the forest industry but also positively impact on the environment and the world"s natural forests.

S4.3

BASIC KNOWLEDGE EVOLUTION THROUGH TREE BIOTECHNOLOGY RESEARCH

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Most of our basic understanding about the regulation of the molecular regulation of plant growth and development has been achieved through research in the plant model species *Arabidopsis thaliana*. Although this is a very powerful model system for most basic aspects of plant biology, it is limited by the fact that *Arabidopsis* is a rapid flowering annual plant. This means that many aspects of plant growth and development which are typical for forest trees can not be efficiently studied in the *Arabidopsis* system. Such aspects include, among others, the cycling between growth and dormancy, the cycling between vegetative and reproductive growth, the very long juvenile phases typical of trees and many aspects of the regulation of wood formation. The central question is to what extent these "tree specific" traits are controlled by "tree specific" genes or if the difference in growth behaviour and life history of annual and perennial plants is caused by a different utilization of the same basic genetic machinery. It is also clear that an understanding of the genetic background to these "tree specific" traits is central for many tree biotechnological applications, which are most often targeting these specific traits. The completion of the poplar genomic sequence provides us with a powerful tool to answer the question about what makes a tree a tree. Parallel comparative genomic research in *Arabidopsis* and poplar including testing of functional conservation of gene activity in transgenic and mutant plants allows us to not only answer questions of great importance for tree biotechnology applications, but also to provide important basic information about plant biology. I will provide a few examples of how parallel work in the *Arabidopsis* and poplar model systems can help to answer basic questions about the regulation of flowering, growth and dormancy and wood formation in trees and how this knowledge can lead to new biotechnological applications.

S4.4

AN ASSOCIATION GENETICS AND FUNCTIONAL GENE TESTING PIPELINE FOR TREE IMPROVEMENT

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The huge cumulative effect that small-effect quantitative trait loci (QTL's) can have on traits of interest is becoming increasingly apparent. However, long generation times of species such as *Pinus radiata* render the use of such loci difficult for two main reasons. First, the pedigrees in *P. radiata* are often only two or three generations deep. Therefore, linkage disequilibrium tends to be maximised. Second, applying knowledge of QTL data via the multigenerational breeding required to bring beneficial loci together is impractical for rapid tree improvement. Therefore, at the Cellwall Biotechnology Centre, we have developed a candidate gene screening and testing pipeline utilising association genetics, in conjunction with gene discovery, functional analysis and transgenic methodologies to circumvent these difficulties. Gene discovery methods are used to identify candidate genes to define points of attack for association genetics. Functional analysis on positive candidates is carried out in a variety of systems with a full battery of analytical methodologies. We have developed a transformable *P. radiata in vitro* tracheary element system which allows the rapid screening of candidate genes for effects on secondary cell wall deposition. Transgenic model plant systems as well as *P. radiata* and other tree transformation systems are utilised to further assess genes for beneficial effects on traits of interest prior to field trials and deployment. Case studies highlighting various aspects of the pipeline will be discussed.

S4.5

UNBLOCKING THE OBSTACLES TO OPEN USE

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Past IUFRO presentations have recognized great potential for using biotechnology innovations in forest plantings to solve problems, ranging from processing industry costs to reclamation of eroded sites to providing increased fuel content (the major use of wood worldwide). However, much work at research institutions, even work that might support wider public acceptance (e.g. up- or down-regulation of endogenous pathways) may never reach end users, and even the most well-coordinated and technically advanced R&D programs are faced with significant challenges to commercialization. Both for clonal forestry and for transgenics, use of the discrete technologies embodied in or used to select and produce the trees may require many intellectual property licenses, and public acceptance concerns represent another freedom to operate risk. For transgenics, adding regulatory timelines dictated by the life cycles of forest trees, the results is likely to be few commercializations, only for a few of the highest margin products. How can we unblock the pathway between valuable research and the end users? Some of the big obstacles can be mitigated by willingness to innovate not only in biotechnology inventions, but in how they are protected and distributed. CAMBIA has long licensed GUS technology widely, and is now employing a new license mode for what experts in the field recognize as a broad work-around of *Agrobacterium*-mediated transformation, with modified plasmids and methods to mediate gene transfer to diverse plant families. We anticipate this will lead to better understanding and use of naturally evolved bacteria-plant interactions; a variety of analyses confirm integration of single or few T-DNA copies into the plant genome, and support further modifications to facilitate mobilization into plants using non-phytopathogenic bacteria while removing lateral transfer functions between bacteria. This technology is not in the public domain. Any research institution or company can have a royalty-free license to use this technology -- but only by agreeing to share, with others that agree to the same conditions, information on the safety of the technology and improvements, and not to assert derivative intellectual property rights against each other (thus creating a protected commons of enabling technology). In the IT industry, such "open source" licensing has spawned successful businesses large and small, and significant profits. Open source licenses are now being applied and applauded for a wider variety of intellectual property, particularly in countries such as South Africa and Brazil. In tree improvement worldwide, similar intellectual property systems have long been in use-- CAMBIA is the first of many, we hope, to employ such a model in biotechnology. In IT, an industry dogged by vulnerability to all sorts of worms, companies that use open source licenses have attracted user engagement in improvement and implementation, and little hacker attack. In the model exemplified by the Apache server, the technology has been adapted to many environments, while supporting a positive public image and a community valuing those who invent. Can we imagine that for our industry?

www.biosnet.~~org~~

www.biolicense.net

S4.6

ADOPTION OF FOREST BIOTECHNOLOGY WORLDWIDE: PROCESS, IMPLICATIONS, AND SOCIETAL ISSUES

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In coming years, application of biotechnology to trees and forests worldwide will steadily yield new knowledge, expanded forest productivity and characteristics, measurable economic and industrial gain, and – it is assumed – societal benefit. Forest biotechnology will also yield a complicated array of societal issues: environmental, ethical, policy, cultural, and economic. The issues will spring of course from the profound importance of trees to culture and place, to emotion and environment. Issues and questions will also arise, not surprisingly, from uncertainty about the safety and value of more targeted alterations to a plant so important to life on this planet. Understanding and addressing the implications of forest biotechnology requires attention not just to science and industry goals, but also an understanding of the significant change at hand: forest biotechnology will be shaped more as a *technological enterprise* than as a more traditional forestry endeavour. As such, understanding the process and components of biotechnology development is required. Merging the reality and implications of this process with the connotations and value of trees offers a framework to consider the questions at hand: about varied representative issues; about responses to plantations of biotechnology-changed trees; about individual and cultural responses to environmental, ethical, and policy considerations; about the implications of *not* developing forest biotechnology. Among recognitions and outcomes of carefully applied forest biotechnology, one seems to appropriately merge cultural imperatives, industry goals, increasing demands for products, and realistic strategy: developing the Trees of Technology to better preserve land, the environment, and indeed the Trees of Tradition.

S4.7p

ACCOMPLISHMENTS AND CHALLENGES OF CONIFER SOMATIC EMBRYOGENESIS FOR THE IMPLEMENTATION OF MULTI-VARIETAL FORESTRY

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Multi-varietal forestry (MVF) may be defined as the use of tested varieties in plantation forestry. The implementation of MVF offers many advantages, including: (1) obtaining a much larger genetic gain per breeding cycle than is possible from conventional seed orchard breeding, (2) flexibility to rapidly deploy suitable varieties with changing breeding goals and environments, and (3) ability to manage genetic gain and diversity in plantation forestry. Despite these advantages, MVF in conifers has rarely been practiced because of the general lack of an efficient vegetative propagation system that can mass-produce the same varieties consistently over time. Recently, owing to the achievements and refinements in somatic embryogenesis (SE) technology, the implementation of MVF with several conifer species has become an important alternative to conventional plantation forestry. The successful implementation of MVF requires three critical phases: (1) An efficient vegetative propagation system must be developed. Conifer SE is the primary enabling technology for all conifer biotechnology products including those for implementing MVF. Currently, SE for most spruce species is sufficiently refined for use in MVF. However, SE for many pine species remains difficult to obtain. In the past three years, laboratories in Canada and France collaborated to improve SE in pines. We report the current state of SE initiation in commercially important pine species and discuss the challenges and possible alternative solutions. (2) High-value varietal lines must be developed. This involves efficient field-testing of embryogenic lines while maintaining them in cryogenic storage. The numbers of families and clone lines within each family are important considerations for optimal exploitation of genetic variability. (3) Varietal lines must be deployed prudently to balance genetic gain and plantation diversity. We discuss various deployment options for MVF.

S4.8p

TOWARDS TRANSGENIC *EUCALYPTUS* TREES IN FOREST PLANTATIONS

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CBD Technologies has discovered and developed a novel modality for altering plant traits by modifying the plant cell wall. The Company's cell wall modulation platform evolved from research on cellulose binding domains (CBDs), which are families of proteins that nature designed to bind to cellulose to facilitate its processing and recycling. CBD Technologies has utilized these proteins to enhance cellulose production and fiber quality in forest trees and is also developing chemicals based on CBD proteins for use in paper manufacturing and modification. The Company has entered into several development and commercialization agreements. The first commercial species that the Company is developing is *Eucalyptus*. The Company has already succeeded in transforming its genes into numerous commercial *Eucalyptus* clones of its industrial partners and initiated a first field trial in 2003. The Company has formed a strategic partnership with the Brazilian group, Suzano Bahia Sul Papel e Celulose S.A., one of the largest integrated forestry, pulp and paper producers in South America and a development agreement with Tree Tech, a member of the AA Alliance Group in Thailand. By assisting partner forestry companies to significantly improve the yields of their sustainable plantation forests, the CBD technology can help to meet growing fiber demand, without resorting to accelerated destruction of native forests. In addition, rapidly growing forests, utilizing CBD-modified trees are capable of sequestering more CO_2, thus providing additional environmental and potential economic benefit to forestry companies. The challenges of forestry biotechnology companies in changing markets will be discussed.

S4.9p

ASSESSMENT OF DIRECT POLLEN DISPERSAL IN *CRYPTOMERIA JAPONICA* USING PATERNAL INHERITANCE TRAIT AND SSR MARKERS

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In Japan, many conifer species including Japanese cedar (*Cryptomeria japonica*) are economically important woody plants, and transgenic biotechnology in addition to traditional breeding will be effectively applied for tree improvement. However, one of major problems associated with the release of genetically modified plants impacts on surrounding natural population via pollen dispersal. A number of population genetics studies have revealed that pollen mediated gene flow from conifer trees widespread dispersal. However a few study of pollen dispersal under the experimental field condition was not consisted with knowledge of population genetics studies, and pollen dispersal observed up to 30m distant from pollen source. We presumed that inconsistency was caused by restricting sampling to both seeds and mother trees. To evaluate the potential gene flow from genetically modified trees, it is necessary to know pollen dispersal distance effective to crossing. Wogon-Sugi is a horticultural variety of *C. japonica*, and its new shoot is white to yellowish white in spring and change to normal green in late summer. Ohba et al (1971) found that this trait is inherited paternally. Therefore we have directly measured pollen dispersal from Wogon-Sugi family. Forty-six Wogon-Sugi individuals were planted at two sites in Forest Tree Breeding Center. Most individuals were at one site. From this site 103 individuals planted at 30 sites within a radius of 300 m distant were used as mother trees to collect seeds. We collected 330,000 seeds (estimated by the weight), and sowed them in April, 2004. A total of 46,017 seedlings germinated and the rate of germination was 14%. In July, 13 Wogon-Sugi type seedlings were observed. After determining their SSR genotypes, actual transmission of the pollen of Wogon-Sugi was confirmed and each pollen parent individual in the Wogon-Sugi family was specified. Their distances from the Wogon-Sugi ranged from 100m to 500m, and the highest frequency, 0.0062 at 300m, was observed south of the central pollen source. The occurrence of seedlings from Wogon-Sugi was very low in this experiment, and it did not decrease significantly with distance from the pollen sources. To avoid yearly fluctuation and confirm in large scale over one million seeds were sown in this spring.

S4.10p

TRANSFORMATION OF COMMERCIAL *EUCALYPTUS* CLONES

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We are developing unique technology for crop and fiber enhancement mediated by cell-wall modification genes. The deployment of our technology for the forest plantation is being realized via transformation of clonal *Eucalyptus*. The transformation of non-juvenile plant material is a challenging task. The *Eucalyptus* explants of clonal material maintain mature properties of their mother tree. In addition, the genetic variation between the different clones of the same species dictates development of a wide range of protocols for different clones. We have developed efficient tissue culture methods for the transformation and regeneration of clonal *Eucalyptus*. We have succeeded in transforming a number of commercial *Eucalyptus* species, including elite *E. camaldulensis*, *E. grandis* and several commercial important *E. grandis* hybrid clones. We have established regional collaborations with leading forestry companies to develop high-performing *Eucalyptus* germplasm for the plantation forest industry. Our first field test with transgenic *Eucalyptus* was planted in 2003 in Israel. This experiment includes several hundred trees expressing the *cbd* & *cel1* genes under different promoters. Additional experiments are underway in commercial territories.

S5. Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

S5.1

SEQUENCE DIVERSITY IN PLANTS: IS THERE FUNCTIONAL VARIATION BEYOND SNPS?

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The extensive lack of colinearity among allelic maize genomic segments has recently been described. Two types of phenomena are responsible for this observation: presence/absence of LTR-retrotransposons and presence/absence of genic fragments. Retrotransposons that are not shared between inbred lines appear to have inserted into the maize genome significantly more recently than those that are shared, revealing an active movement of high copy number elements in very recent evolutionary times. The genic fragment polymorphisms are also due to recent insertions of non autonomous elements of the helitron class as well as of the CACTA class. It has often been postulated that transposable elements are still actively reshaping genomes: the maize genome is in constant flux in that transposable elements continue changing both the genic and non-genic fraction of the genome, profoundly affecting genetic diversity. In addition to the non colinearity we have detected extensive cis-regulatory variation in maize genes, including what we called expression overdominance. We will discuss the implications of the allelic non colinearities for regulatory variation, heterosis and evolution of novel gene functions. We will examine the possibility that similar mechanisms for the generation of sequence diversity are active in other plant species.

S5.2

TOOLS AND STRATEGIES FOR IDENTIFYING CANDIDATE GENES FOR COMPLEX TRAITS IN *POPULUS*

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Dense genetic maps comprised of markers with unique sequence tags, including simple sequence repeats (SSR) and single-nucleotide polymorphisms (SNP), are invaluable for assembling whole genome shotgun sequence data into chromosomal scaffolds. Furthermore, genetic maps provide a means for linking phenotypes to molecular polymorphisms through QTL analysis followed by examination of candidate genes in associated genomic intervals. We have identified and characterized over 2 million SNPs and 63,000 microsatellites in *Populus*. We have mapped over 700 SSR to provide a chromosome-scale view of the *Populus* genome sequence, and we have transferred this map to multiple pedigrees to facilitate extension of the sequence resource throughout the genus. We are now developing a microarray-based SNP genotyping platform to map the vast majority of the sequence fragments that are not yet associated with chromosomal scaffolds. This work will greatly facilitate the application of the genome sequence in identification and assessment of candidate genes underlying complex traits in natural and managed systems. Multiple examples will be provided where initial candidate gene lists are generated from QTL intervals, and these lists are progressively narrowed based on a variety of evidence sources. This includes homology-based inference of gene function, whole genome transcript profiling, and correspondence of gene content of pairs of QTL and introgressed genomic fragments from homeologous chromosomal segments.

S5.3

MOLECULAR INSIGHTS INTO THE GENE POOL OF *EUCALYPTUS GLOBULUS*

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Eucalyptus globulus is one of the most widely planted eucalypts in temperate regions of the world. Its centre of origin is south-eastern Australia, including the island of Tasmania, the southern coast of continental Australia and intervening islands. We have been using nuclear and chloroplast DNA markers to study the genetic architecture of the native gene pool of *E. globulus* and its evolutionary history. Nuclear microsatellite markers have shown that the gene pool is hierarchically structured, with localised genetic variation associated with limited gene flow, superimposed on broad-scale geographic differentiation. While there is some concordance between estimates of genetic divergence between populations based on molecular markers and quantitative traits, there are also significant differences in some cases, possibly due to directional selection acting on quantitative traits. Variation in the maternally inherited cpDNA in *E. globulus* provides a different perspective on genetic diversity in the species. Chloroplast DNA variation is even more structured than that observed with microsatellites, and provides evidence of past migration routes and distribution patterns associated with past climatic oscillations. There is strong evidence that *E. globulus* has captured chloroplast haplotypes from co-occurring species through repeated hybridisation. The impact of this hybridisation on nuclear DNA diversity in the species is now the key question.

Jones 2005

http://members.forestry.crc.org.au/eucalyptus

Steane et al. in press

McKinnon et al 2005 Molec. Ecol.

S5.4

ASSOCIATION GENETICS IN PINE – THE ADEPT2 PROJECT

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Identification of specific genes and alleles controlling naturally occurring phenotypic variation of complex traits provides an understanding of trait genetic architecture, an indirect selection tool for tree breeders, and targets for genetic modification. The ADEPT2 projects is aimed at genetically dissecting complex traits and understand the relationship between naturally occurring genetic and phenotypic variation in forest trees by identifying the relationships between naturally occurring genetic and phenotypic variation in *Pinus taeda* L. A population genomic approach (association genetics) will be applied to associate allelic sequence variation and phenotypic differences. All common alleles will be identified in 5,000 genes and associate at 1,000 loci allelic (SNP) variation with phenotypic variation. Phenotypes will be measured in large structured (discovery) and unstructured (verification) populations that are clonally replicated within and among sites in the south-eastern US, and represent the widest possible spectrum of genetic variation in the species. The traits will include chemical and anatomical wood properties, resistance to disease incited by biotrophic and necrotrophic fungi, gene expression measured in a 14k cDNA microarray, and expression of gene family members using real-time quantitative PCR. To aid interpretation of associations we will measure linkage disequilibrium in ten selected 100kb regions of the genome. We expect to identify the most relevant genes associated with specific wood property and disease related traits in loblolly pine, to be used across pedigrees for marker-aided selection in tree improvement programs.

S5.5

ASSOCIATION STUDIES IN *EUCALYPTUS* SPP

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Linkage disequilibrium (LD) mapping or genetic association studies using natural populations result in higher resolution of marker-trait associations compared to family-based quantitative trait locus (QTL) studies. Depending on the extent of LD, it is possible to identify alleles within candidate genes associated with a trait. Association studies are particularly useful in tree improvement programs as variation in whole population can be analysed and useful alleles of the candidate genes can be identified. Association studies therefore, offer opportunities to utilise within gene variation in tree breeding programs, which is not possible with family-based linkage studies. We have used an open-pollinated progeny trial of *Eucalyptus nitens* (*E. nitens*) to study the effects of candidate genes in controlling wood quality traits. Presence of population structure may confound the results from association studies. To analyse the population structure we have genotyped the *E. nitens* population with 15 microsatellite markers covering the genome. Analysis by the 'STRUCTURE' program indicated that there is little sub-structure in this population. To study the potential of association studies to identify useful alleles of candidate genes we have used *Cinnamoyl CoA reductase* (*CCR*), a key lignin gene. We identified 25 common single-nucleotide polymorphism (SNP) markers in the *CCR* gene in *E. nitens*. Using single-marker and haplotype analyses in 290 trees from *E. nitens* natural population, two haplotypes significantly associated with MFA were found. These results were confirmed in two full-sib families of *E. nitens* and *Eucalyptus globulus*. In an effort to understand the functional significance of the SNP markers, we sequenced the cDNA clones and identified an alternatively-spliced variant from the significant haplotype region. This study demonstrates that useful alleles of candidate genes affecting wood quality traits can be detected using association studies. Results from this study and results from association studies using other candidate genes will be discussed.

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S5.6

TOWARDS A COMPLETE LOBLOLLY PINE GENETIC MAP FOR APPLICATION IN MARKER-DIRECTED POPULATION IMPROVEMENT (MDPI)

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Marker-directed population improvement (MDPI) has been proposed as a molecular breeding strategy for long-lived organisms such as forest trees. The basic concept involves the determination and manipulation of marker-trait associations within subgroups of the breeding population. The marker trait-associations are established by a set of controlled crosses within each subline and determined by marker-trait analyses in the subsequent field trials. The first cycle of selection combines phenotypic and genotypic information to optimize gain given the additive genetic variation. The second and third cycles are completed with genotypic selection only, greatly reducing the time needed to complete these cycles and increasing the genetic gain per unit time. Implicit in this approach is the availability of a complete genetic map of highly informative markers that can be efficiently assayed, and robust accelerated breeding techniques. The recent improvements in top grafting make the later accessible in many pine species and in particular loblolly pine (*Pinus taeda* L.). Our lab is involved in the former in which we are developing and mapping a large panel of microsatellite markers. All markers are screened against a panel of 8 unrelated loblolly pine trees. Those amplifying suitable alleles are selected and tested with polymorphism screening panels for two reference pedigrees. Segregating markers are then mapped to chromosomal bins using one or the other reference mapping pedigree. We will update the status of this project for three sets of microsatellite markers—two developed from cloning and sequencing microsatellite-enriched portions of the loblolly pine genome and the other from mining the current set of loblolly pine ESTs. Our goal for MDPI is to establish 336 polymorphic loci in 112 chromosomal bins.

S5.7

COMPARATIVE MAPPING WITHIN THE GENUS *PICEA* AND ANALYSIS OF SYNTENY WITH OTHER PINACEAE

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The aim of this project was to assemble a composite map for each of *Picea glauca* and the species complex *P. mariana* × *P. rubens* to compare genome structure between phylogenetically distant species in the genus and to enable comparisons with other Pinaceae. For each species, four individual linkage maps were initially constructed from two crosses sharing one parent in common. Codominant markers such as ESTPs and SSRs were positioned onto the individual linkage maps and allowed the construction of conspecific composite linkage maps each integrating about 100 anchor markers. Twelve major linkage groups, corresponding to the haploid chromosome number in *Picea*, were recovered for each species from the placement of 802 markers (714 AFLPs, 38 SSRs, and 50 ESTPs) for the composite map of *P. glauca* and from the placement of 1,124 markers (1,014 AFLPs, 3 RAPDs, 53 SSRs, and 54 ESTPs) for the composite map of the species complex *P. mariana* × *P. rubens*. Additional anchor makers were also positioned onto the composite map of *P. abies* in order to increase the number of interspecific comparison points in the genus. As expected 12 homeoelogous linkage groups were identified among the three *Picea* species composite maps. Synteny was well conserved except for three anchor markers. Markers in synteny were also found colinear. Inter-generic comparisons between the composite maps of *Picea*, *Pinus*, and *Pseudotsuga* identified one major breakdown in synteny, where one linkage group homeoelogous to both *Picea* and *Pinus* corresponded to two linkage groups in *Pseudotsuga*. Implications for the evolution of the Pinaceae genome will be discussed.

S5.8

GENETIC ANALYSIS OF COMPLEX TRAITS IN WILLOW

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The primary objective of the UK Biomass for Energy Crop Improvement Network (BEGIN) is to provide improved biomass willow cultivars for use as a renewable and carbon neutral energy resource. To achieve this goal, molecular genetic and genomic approaches are being utilised to gain further understanding of the genetic basis of important agronomic traits, and also to increase the efficiency of the breeding process through marker-assisted approaches. A two-site mapping study based on a 480 progeny of a cross between two *Salix viminalis* x *S. schwerinii* hybrid sibs has revealed several robust QTL governing total yield, associated yield parameters (e.g. stem height and diameter) and resistance to *Melampsora* rust. Further characterisation of these loci using fine scale mapping and candidate gene approaches is now underway. In addition, the potential to exploit the increasing amount of resources available for poplar is also being explored through comparative studies. The current status of this research and future perpectives will be presented.

S5.9p

DEVELOPMENT OF A SSAP (SEQUENCE-SPECIFIC AMPLIFICATION POLYMORPHISM) MARKER SYSTEM IN MARITIME PINE (*PINUS PINASTER*)

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Sequence-Specific Amplification Polymorphism (SSAP) is a retrotransposon-based molecular marker system that results in the amplification of the region between a retrotransposon-specific primer originating from the LTR region of the retrotransposon and a nearby cleaved restriction site, to which an oligonucleotide adaptor has been added. This technique displays individual retrotransposon insertions as bands on a sequencing gel, however its application requires previous knowledge on the sequence of LTR regions of retrotransposon elements. For copia-like retrotransposons, PCR fragments between a RNaseH conserved motif and a restriction site in the adjacent 3' LTR sequence were isolated by a two-step PCR using degenerate primers following a strategy by Pearce et al. (1999). The mixed PCR products obtained were cloned and sequenced to identify the population of RNaseH-LTR terminal sequences. In gypsy-like retrotransposons, the position of the RNaseH gene does not allow the application of the above-described procedure to isolate LTR sequences and therefore other strategies were followed. An initial sequence of 200 bp (putative *gypsy* -like retrotransposon) has been extended by genome walking in order to reach the LTR regions. Identified LTR sequences were then used to design primers for SSAP analysis. From the several procedures tested for the establishment and optimization of SSAP, the best results were obtained by using IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis (Li-COR Biosciences) followed by gel electrophoresis in the sequencing apparatus LI-COR Global IR^2 system (4200 DNA Analysis System). The developed marker system will be potentially useful for biodiversity studies in *P. pinaster* and it is being applied to compare retrotransposon profiles during *in vitro* propagation in order to study the effect of the propagation method on the activity of retrotransposons.

Pearce S, Stuart-Rogers C, Knox M, Kumar T, Ellis N, Flavell A (1999) Rapid isolation of plant Ty1-copia group retrotransposon LTR sequences for molecular marker studies. Plant J 19: 711-717

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S5.10p

PPRT1 (*PINUS PINASTER* RETROTRANSPOSON 1): THE FIRST GYPSY-LIKE RETROTRANSPOSON ISOLATED IN *PINUS PINASTER*

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The retrotransposons form an important and ubiquitous class of mobile genetic elements, which, like retroviruses, propagate intracellularly through transcription and translation via an RNA intermediate, followed by packaging of the transcript into particles, being non-infectious. They are divided into two classes based on their structure: the retrotransposons with long terminal repeats (LTRs) and the nonLTR retrotransposons. Retroelements with LTRs have been characterized and named according to the *Drosophila* type elements, as either *copia*- or *gypsy*-like, based both on the order of their protein coding domains found between the long terminal repeats (LTRs) and on their sequence similarities. Here, we report the isolation of a *gypsy*-like retrotransposon complete sequence from maritime pine, named *PpRT*1 (*Pinus pinaster* retrotransposon 1). The first short sequence (251 bp) of this retrotransposon was initially isolated in INRA (France) as a result of an EST project in maritime pine. The *PpRT*1 retrotransposon is a LTR element that shares features with the gypsy retrotransposons and strong similarities with *IFG*7, an active retrotransposon found in *Pinus radiate*. We found that *PpRT*1 was 39 bp longer than *IFG*7 and the whole sequence shares 95% similarity. Our hypothesis was that *PpRt*1 is an active element being able to transpose and present in the genome with several copies. We found that this particular retrotransposon is a low copy number element and up to now we have not found any evidence of activity and transposition in the maritime pine genome, even in stressful conditions. This kind of sequences will be potentially useful in molecular markers strategies.

S5.11p

ANALYSIS OF MARITIME PINE ADAPTATION TO CHANGING ENVIRONMENTAL CONDITIONS

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Changing environmental conditions affect drastically development and productivity of long-lived organisms such as pine species. Survival of this tree species is associated with their ability to adapt to new environmental conditions, which depends on the genetic variability of genes involved in control of adaptive traits, and on their plasticity. We aim to understand maritime pine (*Pinus pinaster* Ait.) capacity to adapt to environmental changes such as drought stress and light response following a multidisciplinary approach that integrates experts on molecular biology, population and quantitative genetics and ecophysiology. We are analysing candidate genes involved in fitness-related traits such as bud phenology and drought stress response, to quantify their variability in natural populations compared to variability found at putatively neutral loci (including microsatellites and DNA sequences) in a provenance-progeny test of *P. pinaster*, which represents the accused rainfall cline of the species. This study will help to determine intra- and inter-population genetic variability and distinguish adaptive variation from variation due to historical processes, estimated from association studies between genotypic and phenotypic variants.

S5.12p

TILLING AND MUTATION DETECTION IN *EUCALYPTUS GLOBULUS* SSP *GLOBULUS* FOLLOWING EMS TREATMENT OF POLLEN

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Tree species do not lend themselves to traditional mutation induction methods because of their long reproductive cycles and an exponential need for land and other resources as trees mature. However, it may be possible to combine molecular mutation detection technologies with pollen mutagenesis to generate and identify trees with mutations in selected genes in a relatively short period of time. To test this hypothesis, we trialed pollen mutagenesis in *Eucalyptus globulus* by pollinating four ramets of a commercial clone (provided by Grand Ridge Plantations, Victoria, Australia) with a mix of pollen from six trees that had been treated with the chemical mutagen ethyl methanesulfonate (EMS). Initial screening associated pollen treatment with a significant reduction in seed set. Following germination of seed derived from pollinations using mutagenised pollen, DNA was extracted from 192 seedlings for molecular mutation detection. Three *E. globulus* gene fragments, representing cinnamyl alcohol dehydrogenase (CAD), *Eucalyptus* LEAFY (ELF) and alpha tubulin (alpha-TUB), respectively, were used in a TILLING approach (Colbert *et al.* 2001, Plant Physiol. 126:480-484), revealing the presence of ten inherited, discreet, polymorphic regions in each of the CAD and ELF amplicons that were screened and 13 polymorphic regions in the alpha-TUB amplicon. Twenty of these polymorphic regions were subsequently associated, via sequencing, with 17 single nucleotide polymorphisms (SNPs) and three single-basepair insertion-deletion polymorphisms (INDELs). Screening of 403740 basepairs of *E. globulus* DNA uncovered a total of 1509 inherited polymorphisms and, after elimination of pollen/seedling contaminants and false positives, detected a single, confirmed induced mutation in the alpha-TUB amplicon, which, however, did not alter the predicted amino acid sequence. These initial results illustrate that combining pollen mutagenesis and molecular mutation detection in trees provides a promising approach for generating and identifying mutations in carefully selected candidate genes.

S5.13p

GENOME AND QTL MAPPING OF TRAITS RELATED TO PRODUCTIVITY AND ADAPTATION TO CLIMATE CHANGE IN SPRUCE

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Black spruce (*Picea mariana*) and red spruce (*Picea rubens*) provide an ideal species pair for conifer genomics work. One of the objectives of our comparative structural and functional spruce genomics program is to construct high density to saturated genetic maps of black spruce and black spruce x red spruce hybrids, and to map genetic factors controlling traits related to growth and adaptation to climate change. We have developed a super high density and complete genetic linkage map of black spruce using a three-generation outbred pedigree and that of black x red spruce hybrid using an interspecific BC1 pedigree. The black spruce map has 941 markers distributed over 12 linkage groups (*Picea*, n=12), and covers 1898 cM of the map length, with an average of 1 marker every about 2 cM. The black x red spruce hybrid map has 1216 markers distributed over 12 linkage groups, covering 1865 cM, with an average distance of 1.5 cM between adjacent markers. The estimated genome size of black spruce is about 1900cM. We are mapping candidate genes and QTLs for growth, adaptation to elevated CO_2 levels, water use efficiency and other ecophysiological adaptive traits using replicated clonal genetic tests of the mapping populations under different abiotic stress conditions.

S5.14p

CHARACTERIZATION OF CANDIDATE GENES FOR WOOD PROPERTIES IN *EUCALYPTUS*

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Co-localization of QTL and putative Candidate Genes (CG) will allow the selection of CG with increased chances of functional association. However, additional characterization criteria are necessary in order to restrict the number of putative CG to map in genetic linkage maps. Together with a South European consortium integrating industrial (RAIZ and ENCE) and academic partners (UMR CNRS-UPS, CIRAD-Fôret) we have constructed three normalized SSH cDNA libraries (*E. gunnii* xylem *vs* leaves, xylem *vs* phloem and *E. globulus* juvenile *vs* mature) used to put together a eucalypt wood unigene set. Through the *Genolyptus* consortium we have currently access to 91,000 EST sequences from ten (non-normalized) cDNA libraries from different eucalypt tissues (e.g. xylem, phloem, leaves) of several species (e.g. *E. grandis*, *E. urophylla*, *E. globulus* and *E. pellita*). We have performed BLAST sequence homologies to the *Genolyptus* database. Preliminary results indicate that around 80% unigenes have homologous sequences in the *Genolyptus* databases: about 15% of these were exclusively found in xylem libraries, 15% were completely absent from xylem libraries and 50% were common to several libraries. The 20% remaining sequences may correspond to genes represented with non-homologous fractions (EST) in the two databases or to low-expressed genes, only represented in the European databases. This database comparison approach is a valuable tool both to improve the functional characterization of the unigenes and to check if the SSH procedure was effective in revealing low expressed genes.

S5.15

NUCLEOTIDIC VARIABILITY OF THE CCR GENE IN *EUCALYPTUS UROPHYLLA*

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The use of candidate gene (CG) in marker-aided selection relies to the study of allelic variation in breeding population. Single nucleotide polymorphism (SNP) offer new perspectives to describe the functional variability of CG within breeding or natural populations. The aim of our study was to describe the nucleotidic variability within the cynnamoyl CoA reductase (CCR) gene, a structural gene of the lignin biosynthesis pathway, within a representative sample of *Eucalyptus urophylla*. 40 *E. urophylla* genotypes have been selected representing 4 provenances from the Flores island. Seven DNA fragments (around 600 bp) were sequenced by direct sequencing of the PCR product and a statistical method (PHASE software) was used to infer phase and to reconstruct haplotypes, using genotype data. Most of probable haplotypes were reconstructed with a high probability (0.9). The analysis of nucleotidic variability was carried out for 82% of the CCR gene. A high level of variability has been detected both in exons and introns with mainly neutral variation. Nevertheless, 5 non-neutral mutations have been detected in exon 4 and 5, but these mutations do not affect known functional protein sites.

S5.16p

COMPARATIVE ANALYSIS OF SNP MARKER DIVERSITY IN LIGNIN BIOSYNTHETIC GENES OF *EUCALYPTUS GRANDIS* AND *EUCALYPTUS SMITHII*

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Single nucleotide polymorphism (SNP) markers can be used to analyse allelic diversity in candidate wood formation genes in forest tree populations, to study differentiation among populations and species at the locus level and, ultimately, to establish marker-trait association for marker-assisted breeding purposes. The aim of this pilot study was to compare SNP marker diversity in South African breeding populations of two *Eucalyptus* tree species, *E. grandis* (a subtropical species) and *E. smithii* (a temperate species). SNP markers were identified by sequencing DNA fragments of two lignin biosynthesis genes in a SNP discovery panel consisting of 20 selected *E. grandis* and 20 *E. smithii* individuals. Two DNA fragments (~2000 bp in total), representing the 5' and 3' ends of 40 cloned alleles each, were sequenced for the *CAD2* (cinnamyl alcohol dehydrogenase) gene, a structural gene in the lignin biosynthetic pathway, and the *LIM1* gene, which encodes a transcriptional regulator of *CAD2*. Overall SNP density in *CAD2* was one SNP per 51 bp in *E. grandis* and one per 58 bp in *E. smithii*. Much lower SNP density was observed for *LIM1* in *E. grandis* (one in 155 bp) compared to a density of one SNP per 45 bp in *E. smithii LIM1*. Linkage disequilibrium (LD) among SNP sites generally decayed across the length of the gene, suggesting considerable historical recombination. We constructed two SNP marker panels (for use in the Applied Biosystems SNaPShot Multiplex kit) to successfully tag all the major allele groups of each gene. The *CAD2* SNP panel consisted of four (shared) interspecific, three *E. grandis*-specific and four *E. smithii*-specific SNP markers. The *LIM1* SNP panel consisted of four shared SNP markers and five SNP markers specific for each species. The ability of the SNP marker panels to detect all observed haplotype groups as well as additional SNP haplotypes was tested in 100 *E. grandis*and 140 *E. smithii* individuals sampled from first-generation selections of the two species. This will allow us to study the differentiation and phylogeographic distribution of SNP haplotypes in these two distantly related eucalypt species.

S5.17p

CHLOROPLAST DNA DIVERSITY IN TEMPERATE AND TROPICAL *SALIX* SPECIES

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Salix is a large genus of woody plants with wide distribution and significant contribution to the natural dynamics of floodplain ecosystems. In this study, we investigated the chloroplast DNA diversity in *S. alba* and *S. fragilis* which are dominant species along several European rivers as well as *S. subserrata* which is widely distributed in Africa and *S. murielli* from Sudan. Several cpDNA markers were used (targeted PCR-RFLP, point mutation specific nested primers, primers designed for INDELs). Inter and intra-specific mutations between *Salix* were due to the presence of both INDELs and SNPs. Only one haplotype was obtained for *S. fragilis* with no geographic distribution and three for *S. alba*. *S. alba* had three haplotypes with strong geographic distribution in Europe ($G_{ST\ 0.88}$).The two African *Salix* species had no geographic distribution along the Blue and the main Nile regions investigated due to the shared presence of both species cpDNA haplotypes in six out of seven populations. For the African species, each species was represented by one cpDNA haplotype. We conclude that in both temperate and tropical *Salix* species, no shared haplotypes were detected. Moreover, both the European and African *Salix* species displayed low cpDNA genetic diversity compared with other tree species.

S5.18p

A STRATEGY TO IDENTIFY MUTATIONS OF ADAPTIVE SIGNIFICANCE IN MARITIME PINE

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Long-lived organisms such as forest trees will be greatly affected by the rapid climatic changes predicted for the near future. A greater rate of temperature increase associated with a higher heterogeneity in rainfall may constitute stronger pressures of selection, compromising their long-term adaptation comparing to annual plants with higher reproduction rate. In order to assess tree species' adaptive potential, one possible approach is to focus on the levels and structure of genetic diversity of genes involved in fitness-related traits like water stress tolerance. In this study, we are developing a four-phase strategy to identify mutation of adaptive significance for water stress response in maritime pine. First, provenance test is established in France base on open pollinated progeny of 600 mother trees sampled throughout the natural range of the species, corresponding to populations receiving from 350 to 1400 mm rainfall. Growth, water used efficiency and a series of ecophysiological parameters will be used to measure the adaptive potential of the trees. Second, a list of candidate genes was proposed based on literature result and ongoing gene discovery experiments where the accumulation of transcripts and proteins is measured facing water deprivation. Third, we described the landscape of nucleotide diversity in this genes (type of mutation, level and structure of diversity, extent of linkage disequilibrium, departure from neutrality and/or demographic equilibrium, and type of selection) with the main objective of identifying relevant mutation (i.e. that natural selection has favoured). In the fourth step, validation of functionally important SNPs is performed in associating nucleotide diversity with the phenotypic variation of adaptive traits.

S5.19p

EVALUATION OF GENE FLOW BETWEEN EXOTIC AND NATIVE TREE SPECIES; DEVELOPING AN ALTERNATIVE METHOD FOR MEASURING THE IMPACT OF THE INTRODUCTION OF GENETICALLY MODIFIED TREES ON THE INTEGRITY OF LOCAL POPULATIONS

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Estimation of the levels of effective gene flow towards native species represents the first step in the assessment of ecological risks linked to the introduction of GMO trees into the environment. In order to evaluate the risks of such gene flow, several authors have recently suggested assessing spontaneous introgression levels using non-GMO exotic material already established in the field. In this study, we use species-specific markers to monitor the rate of gene flow from plantations of non-GMO exotic species of *Larix* and *Populus* into natural populations of their respective native congeners. For *Larix*, we use primer extension and CAPS techniques to genotype paternally inherited chloroplast markers and maternally inherited mitochondrial markers, allowing for a rapid identification of first generation hybrids. For *Populus*, we use SNPstream technology to simultaneously genotype 12 nuclear, species-specific, SNPs, allowing for a high power of identification of first and second-generation hybrids. Preliminary results for *Larix* show that the rate of hybridization is moderate: genotyping 1779 offspring produced by 98 trees of native *L. laricina*, we found that 2.2% of the offspring exhibited exotic gene contributions. A rate of gene flow of this order of magnitude may have a significant impact on the genetic integrity and diversity of the native tree species, depending on population sizes and possible fitness effects of introgressed genes.

S5.20p

GENOMICS AND DIVERSITY OF BUD BURST IN SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL.)

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Productivity and distribution of long-lived species, such as forest trees, are intimately related to phenological events, especially bud burst, and may be greatly affected by rapid global changes in the near future. With this aim in mind, we are developing a research program focused on the date of bud burst for sessile oak (*Quercus petraea*(Matt.) Liebl.). We followed a transcriptomic approach to identify relevant candidate genes for the trait. SSH libraries were produced from buds harvested during their development from the dormant bud to the elongating shoot. Expression levels of about 800 clones, representing 233 unique transcripts, have been assessed using cDNA macroarray. Regarding expression patterns, we selected several expressional candidate genes. This set of candidate genes, complemented by genes already published, has been mapped for *Quercus Petraea* and *Castanea sativa* (chestnut) and their location compared to those of the QTLs for the trait in both Fagaceae species. A SNP polymorphism investigation have been conducted for seven relevant candidates in order to study associations between nucleotide diversity and the date of bud burst assessed in 21 populations of sessile oak (24 individuals/pop.), originated from the whole natural area and exhibiting contrasted patterns for the trait. Preliminary results for these association studies will be reported here. In summary, this study provided tools to assess diversity of bud burst in natural populations, but also allowed to dissect molecular pathways involved in the control of the trait.

S5.21p

POLYMORPHISM DISCOVERY IN CANDIDATE GENE FRAGMENTS FOR CELLULOSE CONTENT VARIATION IN *PINUS RADIATA* AND *PINUS PINASTER*

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Wood is one the world's most important natural materials and *Pinus* species are of international importance as plantation and natural sources for wood fibre and solid wood. *Pinus radiata* D.Don (radiata pine) is the premiere softwood plantation species in Australia and New Zealand and plays an important role also in Chile and Portugal. *Pinus pinaster* Ait. (maritime pine) is an important commercial species in the southwest of Europe and is the primary coniferous plantation species in France. Modern molecular methods offer the opportunity for more rapid domestication of these species and efforts to understand the genetic control of commercially important traits are underway in both species. With long generation times, population heterogeneity and large genome size hindering the efficient use of many approaches, association studies using candidate genes hold much promise for accelerated domestication. Such approaches rely on the identification of polymorphic sites within candidate regions but accessing and utilising this diversity pose major challenges for researchers. In this poster we present a summary of polymorphism screening results from a set of cellulose biosynthesis candidate gene regions in both radiata and maritime pine. Increasing the number of identified polymorphisms will improve the power and range of molecular applications and increase our understanding of local patterns of linkage disequilibrium in coding regions of pine genomes.

S5.22p

POPULATION STRUCTURE AND ADAPTATION OF EUROPEAN TREMBLING ASPEN IN SWEDEN - A STUDY ON THE SWEDISH ASPEN COLLECTION (SWASP)

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Adaptations to the local environment are important for species with wide habitat ranges and this is particularly true for a long-lived tree species. Local adaptation occurs even in species with extensive gene flow such as European trembling aspen (*Populus tremula*). Ten trees from twelve different provenances throughout Sweden were studied for population structure with neutral molecular markers (single sequence repeats, SSRs). Each individual tree was also cloned and planted in common garden experiments on two sites to facilitate G×E and quantitative trait estimates. Genetic differentiations at the putatively neutral SSR loci indicate slight structuring of the Swedish Aspen population and extensive gene flow among populations. However, a few loci had lower allelic variation than neutral expectations, possibly indicating areas of the genome with either low recombination or linkage to areas under strong selection. Provenance explained the majority of the variation in phenology with QST values above 0.60 for bud set and length of growing season.

S5.23p

MAPPING CANDIDATE GENES FOR WOOD QUALITY IN *EUCALYPTUS UROPHYLLA* AND *E. GRANDIS*

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Within the framework of a South European consortium integrating industrial (RAIZ and ENCE) and academic partners (UMR CNRS-UPS, CIRAD), a candidate gene (CG) approach has been developed for wood quality in *Eucalyptus*. cDNA libraries from *E. gunnii* have been constructed and a eucalypt wood unigene set of 224 genes was available for differential expression analysis during xylem differentiation. A second step of our approach consists of the genetic mapping of expressed sequence tags (ESTs) and the study of co localisation between CG and QTL for wood quality. The aim of the current study was to localize EST on genetic maps of *Eucalyptus urophylla* and *E. grandis* using an interspecific cross of 201 individuals and to test the effect of CG on the variation of wood quality traits. Specific primers were designed for 90 EST of the *E. gunnii* unigene and after optimizing PCR conditions, the detection of polymorphism was realized using the single strand conformation polymorphism (SSCP) technique. A number of 47 EST were mapped for at least one of the two parents. According to their segregation in the progeny, some EST should correspond to duplicated genes. Finally, some co localisations with wood quality traits were detected. Even if these results need to be validated by the study of gene effects within breeding populations, they open up new perspectives for markers assisted selection (MAS) for wood quality. Beyond its interest for MAS, this work allowed the localization of new co dominant markers on eucalyptus genetic maps and provide a basis for investigating genome organisation and identifying synteny among the *Eucalyptus* genus.

S5.24p

CONSERVATION OF XYLEM PREFERENTIALLY EXPRESSED SEQUENCE TAGS IN SEVERAL SUBGENERA OF *EUCALYPTUS* L'Hérit

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Large eucalypt genomic resources, especially expressed sequence tags (ESTs), have been recently developed using a limited number of economically important species of the subgenus *Symphyomyrtus*. The usefulness of these databases to describe and use the huge genetic diversity of the genus relies on the sequence conservation. In this study, primers designed from a set of 19 ESTs isolated from *E. gunnii* xylem (subgenus *Symphyomyrtus*, section *Maidenaria*) were used to amplify putative homologous genes in 30 species of *Eucalyptus* L'Hérit., (nineteen *Symphyomyrtus*, five *Monocalyptus*, two monotypic subgenera *Nothocalyptus* and *Idiogenes* and four *Corymbia*). Specific PCR products were compared between genotypes according to their length. Verification of the amplified fragment identity was performed through direct sequencing of a random sample of PCR products. Based on presence/absence of the expected band, neighbour joining tree were constructed in order to reveal genetic proximity to the reference species. The overall rate of amplification was 81%. Four EST amplified on the 89 tested genotypes. The rate of transferability decreases according to the phylogenetic distance with *E. gunnii*, from 94% for sections *Maidenaria*, *Transversaria* and *Exsertaria*, to 68% for the subgenus *Monocalyptus* and only 48% for the remote subgenus *Corymbia*. The present work shows the high potential of eucalypt ESTs for comparative mapping and association studies between candidate genes and quantitative traits at subgenus or genus level.

S5.25p

PATTERN OF VARIATION OF *EUCALYPTUS UROPHYLLA* FOR ADAPTIVE TRAITS, GROWTH AND MICROSATELLITES

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Understanding the pattern of variation within the natural range of tree species can help to manage the genetic resources for breeding or conservation purposes. We combined microsatellites and quantitative traits to assess genetic diversity of *Eucalyptus urophylla*, one of the most important tree species used in plantation in the humid tropics. Eighteen populations (360 individuals) representative of the main occurrence of the species in Indonesia were analysed with ten microsatellites. Two progeny/provenance trials, corresponding to the same populations (22650 individuals) established in the Congo were analysed for growth and adaptive traits at 37 months. Molecular analyses showed a moderate to high diversity among populations (Hob=0.44-0.71), positive Fis which were likely to result from a whalund effect and a low differentiation parameter Fst=0.04, explained by an effective gene flow by pollen. The genetic variances within and among populations for adaptive and growth traits were significantly different from zero ranging from 13 to 23% and from 14 to 50% of the total variation respectively. A strong negative relationship between altitude of the seed source in Indonesia and the performance in the Congo was observed (R^2=0.59-0.67) and was explained by the effect of the natural selection due to the variation of abiotic factors with altitude. This clinal variation constitute a very good experimental design for studying the variation of candidate gene related to adaptation in natural population, which will be the next step of the study.

S5.26p

IDENTIFYING G x E INTERACTIONS INFLUENCING GROWTH CHARACTERISTICS AND QTL DISCOVERY FOR AN F_2 POPULATION OF HYBRID POPULUS (*P. DELTOIDES x P. TRICHOCARPA*) GROWN AT THREE CONTRASTING SITES - LINKING GENETIC INFORMATION TO THE PHYSICAL SEQUENCE

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Fast-growing hybrid poplar (*Populus* spp.) has potential as a valuable fuel crop for renewable carbon neutral energy. An F_2 population (Family 331; POP1) derived from a cross between *Populus trichocarpa* (93-968) and *P. deltoides* (111-129) was grown at three geographically different sites across Europe. The performance of POP1, based upon main stem traits and biomass, improved from northern to southern Europe. Significant genotype x environment interactions were seen despite heritability values being moderate to high. One hundred and ninety-seven QTL were identified for the traits across the sites, with many collocating for the correlated stem or leaf traits. QTL were also seen to collocate across the different environments. Five linkage groups for biomass were identified as being of particular interest based on QTL hotspots. These hotspots have been analysed in detail, linking to the physical sequence of poplar and several candidate genes for future improvement identified. These will be presented here.

S5.27p

ALLELIC DIVERSITY OF CELL WALL BIOSYNTHETIC GENES IN *EUCALYPTUS UROPHYLLA*

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Eucalyptus urophylla S.T. Blake (Blake, 1977) is endemic to islands of the Lesser Sunda archipelago situated north of the Australian continent. Many of the provenances of *E. urophylla* are very difficult to reach and there is an urgent need to conserve genetic diversity in this tropical species, which is often grown in hybrid combinations with other *Eucalyptus* species, such as *E. grandis*. Over the last decade, the Central America and Mexico Coniferous Resources Cooperative (CAMCORE) collected seed throughout the seven islands that constitute the natural range of the species. A representative sample of this seed is being used to establish an experimental reference population for *E. urophylla* in a large, well-managed and uniform site in South Africa. The aim of the present molecular diversity study is to analyze patterns of nucleotide and haplotype diversity in three wood formation genes (cellulose synthase, *EuCesA1*; sucrose synthase, *EuSuSy1* and cinnamyl alcohol dehydrogenase, *EuCAD2*) primarily involved in cellulose and lignin biosynthesis in *E. urophylla*. This is being achieved by sequencing two DNA fragments of approximately 1 kb from the two ends (5' and 3') of one randomly cloned allele in each of 25 representative individuals. Sequence analysis of more than 2.2 kb of *EuCesA1* sequence revealed very high haplotype diversity (> 0.9), with most of the nucleotide diversity (> 0.009) in introns. Four major allele groups could be identified with some alleles showing possible regions of recombination. DNA sequence analysis of *EuSuSy1* and *EuCAD2* is still in progress and will be included in this presentation. Putative single nucleotide polymorphism (SNP) markers identified for each gene will be assayed in a larger subpopulation (n = 96, made up of two randomly selected families per provenance and several provenances per island) in order to verify SNP markers and obtain better estimates of allelic diversity. These results will be compared to genome-wide patterns of genetic diversity (estimated with microsatellite markers) in order to obtain a detailed overview of genetic diversity in the natural range of *E. urophylla*.

S5.28p

NUCLEOTIDE DIVERSITY AND LINKAGE DISEQUILIBRIUM IN THREE *EUCALYPTUS GLOBULUS* GENES

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Genes that control variation in complex traits can be identified by associating genotypic and phenotypic variation detected in genetic crosses or natural populations. Quantitative trait marker association based on population-wide linkage disequilibrium (LD) is advantageous in that it has higher resolution than traditional mapping approaches. It also requires information about nucleotide diversity and LD structure at candidate genes. To evaluate the feasibility of association genetic studies in *Eucalyptus*, we have sequenced genomic segments from three genes (cinnamyl-alcohol dehydrogenase, *CAD*; ferulate-5-hydroxylase, *F5H*; and S-adenosylmethionine synthase, *SAMS*) in a nucleotide discovery panel of *Eucalyptus globulus* (RAIZ). Genes were tentatively characterized in a set of 17 genotypes that encompass the genetic variation of breeding material in Portugal and a pool of 24 genotypes, originated from 12 *E. globulus* provenances in continental Australia. Target genes were amplified by PCR, cloned and sequenced in a capillary sequencer. After manual sequence assembly and alignment, nucleotide diversity and LD structure analysis were carried out. The nucleotide diversity (pi) was very high (~0.01) for all genes that were analyzed, even when only parsimony informative sites were included. Most important, LD extended in general over 500-1000 bp, indicating that by sampling multiple gene regions (5' and 3' ends) we might be able to identify polymorphisms in LD with quantitative trait nucleotides. Major pitfalls for the analysis of a larger number of genes in many genotypes was the lack of full- or near full-length sequence for the candidate genes in *Eucalyptus*, and the cost and labour required for amplification of individual alleles. Nonetheless, this study supports the feasibility of association studies in *Eucalyptus*, particularly as other alternatives for high throughput genotyping of heterozygous species may become available fairly soon.

S5.29p

MOLECULAR EVOLUTION OF CELLULOSE SYNTHASE GENES IN A SPECIES-WIDE REFERENCE POPULATION OF *PINUS PATULA*

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In terms of area planted, *Pinus patula* is currently the single most important forestry species in South Africa. However, little is known about the molecular basis of intraspecific variation in wood properties of *P. patula*. This study aims to obtain the first estimates of nucleotide and allelic diversity in key wood formation genes of *P. patula*. A reference population of 972 *Pinus patula* trees, consisting of 108 families of 9 individuals each, has been selected for molecular and population genetic analyses. This unique population represents the entire natural range of *Pinus patula* in Mexico and has been fully characterized for physical wood property traits such as wood density and tracheid lumen diameter. Twenty-four trees sampled from the reference population are being used as an allele discovery panel for single nucleotide polymorphism (SNP) marker development in *P. patula*. Preliminary DNA sequence analysis suggests that the overall nucleotide diversity for the *cellulose synthase 1* gene of *P. patula* (*PpCesA1*) is approximately 0.004 (0.4%). The highest nucleotide diversity was found in the UTR regions (1.8%) followed by the intronic regions (0.7%). The haplotypes diversity was very high (100%) and six to seven major allele groups could be distinguished. This study will provide insights into the evolutionary genetics of *P. patula* and provide estimates of nucleotide diversity, gene flow, recombination and linkage disequilibrium in wood and fibre genes of *P. patula*.

S5.30p

QTL DETECTION FOR *MYCOSPHAERELLA* LEAF DISEASE IN *EUCALYPTUS GLOBULUS*

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Mycosphaerella spp. fungal pathogens are a major cause of leaf disease in *Eucalyptus globulus* plantations in Australia and overseas. An F_2 cross was generated and planted in 1998 in order to study the genetic control of resistance to *Mycosphaerella* in *E. globulus*. The four grandparents of the F_2 were chosen on the basis of their divergent breeding values for susceptibility to *Mycosphaerella*. The F_2 consisted of 120 genotypes each represented by two clonal replicates. Phenotypic scores for resistance to *Mycosphearella* and various other traits have been collected annually since the establishment of the trial. The level of *Mycosphaerella* damage in the mapping trial was high and strongly correlated with the level of damage in the same genetic material planted elsewhere on the island of Tasmania, Australia. Parental and consensus maps were constructed using amplified fragment length polymorphism (AFLP) and microsatellite (SSR) markers. The integrated map featured 10 linkage groups and 165 markers, including 33 SSR and 132 AFLP loci, a small 11 th group was identified in the male parent only. The inclusion of SSR markers previously mapped in several different eucalypt species within subgenus *Symphyomyrtus* demonstrated that linkage orders previously reported in *E. globulus, E. grandis* and *E. urophylla* were largely conserved. The two QTL explaining the largest fraction of the variation in resistance to *Mycosphaerella* were located on different linkage groups. The stability of resistance across sites suggests that QTL for resistance will be transferable to other crosses within *E. globulus*.

S5.31p

THE BIOMASS FOR ENERGY GENETIC IMPROVEMENT NETWORK (BEGIN)

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The UK Government is committed to a 10% increase in renewable energy use by 2010 as part of its commitment to reduce carbon dioxide emissions. In order to generate significant amounts of energy from biomass, energy crops will need to be grown specifically for this purpose. A key factor in meeting government targets is the availability to growers of biomass varieties that remain free from pests and diseases and that achieve sustainable high yields. Short Rotation Coppice (SRC) willow is expected to make a significant contribution in this area. Although a range of high yielding willow varieties are now available, great potential for further yield improvement remains. Higher yielding genotypes, with diverse pest and disease resistances and suitability for a wide range of growing conditions, are needed by the UK industry. Under funding from the UK Department of Environment, Food and Rural Affairs (DEFRA), the Biomass for Energy Genetic Improvement Network (BEGIN) aims to meet these requirements through the delivery of a targeted willow breeding programme that is underpinned by molecular genetics and genomics research. Enhancement of the willow genetic map and the identification of robust QTL for traits of agronomic importance have been initial objectives of this project. More recently, the genetic relatedness of willow and poplar is being exploited through comparative analyses of the marker, QTL and genomics data available in both genera. Further characterisation of selected willow and poplar QTL based on positional and functional candidate gene approaches is now underway. The development and use of marker-assisted selections within the breeding programme is also being investigated.

S5.32p

SSR MARKER DEVELOPMENT: WORKING TOWARDS IDENTIFYING QTL'S AFFECTING DORMANCY RELEASE IN APPLE (*MALUS X DOMESTICA* BORKH.)

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Winter temperatures in the Western Cape region of South Africa are not cold enough for normal dormancy release in apple trees during spring. Initial vegetative budbreak has been shown to be associated with prolonged dormancy symptoms experienced by local farmers. Marker-assisted-breeding (MAB) and selection (MAS) of apple cultivars with a lower chilling requirement will be beneficial for local production and will ensure that South Africa remains one of the biggest competitors on the global export market. The first step towards MAB is the generation of a genetic linkage map. In order to generate a reference map, consisting of markers that are transferable between closely related species, the main focus of our research program has been on the development and implementation of microsatellite (SSR) markers. We have developed 310 new SSR markers, using apple and pear sequences, containing di-, tri- and tetranucleotide repeats. Newly developed markers, as well as 193 published markers, are being used for cultivar identification studies. Polymorphic markers are used to screen the progeny of 5 controlled crosses made in order to study and identify quantitative trait loci (QTL) affecting dormancy release after winter. The prediction, optimisation, multiplexing and mapping of the complete set of markers is an ongoing process, and the current status of the program will be described.

S5.33p

DEVELOPMENT AND MAPPING OF EST-DERIVED MICROSATELLITES IN *EUCALYPTUS*

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The Project GENOLYPTUS generated a database of ~120,000 EST and ~40,000 BAC-end and genomic shotgun reads from different species and tissues of *Eucalyptus* with multiple objectives of genomic resource development and functional investigation. Preliminary mining of the EST database was carried out with the goal of having 1,000 optimized microsatellite markers for map enrichment and QTL detection. Clustering of the original EST reads resulted in a 33,500 unigene set involving sequences from different species. With an optimized microsatellite pipeline based on the software MREPS simple sequence repeats were identified in 1,765 consensi under the following parameters: 2-6 bases SSR motifs, perfect structure, i.e. no microvariant interruptions, and a minimum core of 12 bases. Primer pairs flanking these microsatellites were designed for 1,244 markers with Primer3 under the same stringency parameters and allowing expected products between 80 and 350 bp. These 1,244 potential markers included 116 hexa, 51 penta, 82 tetra, 690 tri and 305 dinucleotide repeats. These markers were also classified by the inferred position of the SSR either in the 5'-UTR, ORF or undetermined position based on a BLAST based script against the *Arabidopsis* genome and followed by a preliminary annotation. A first battery of markers was tested involving mainly sequences with high E-value hits to interesting genes to be positioned on the genetic map. Among these genes there are various classes of transcription factors, protein kinases, enzymes involved in polysaccharides and lignin metabolism as well as resistance homologs and heat shock protein. Screening for amplification, polymorphism and interspecific transferability were carried out in a panel of either 12 or six trees used as parents in the GENOLYPTUS mating design. Out of this first battery of 549 microsatellite markers, 419 amplified easily interpretable products and the other 130 either did not amplify or resulted in multiple banding patterns. All 419 microsatellites were transferable among at least five of the six species tested (*E. grandis, E. urophylla, E. camaldulensis, E. globulus, E. dunnii*). From this group of 419 markers at least 266 were polymorphic as evaluated on agarose gels. On re-screening of a set of 48 markers previously classified as monomorphic, 28 displayed polymorphism on high resolution gels indicating that more polymorphic markers could still be derived. Microsatellite markers located in 5'-UTR were significantly more polymorphic than those in ORF ($p=0.0083$ after Fisher exact test). A first set of 167 markers were selected for fluorescent labelling and mapping on the three cloned reference pedigrees involving *E. grandis*, *E. urophylla* and *E. globules*. Microsatellites derived from EST were numbered EMBRA800 and up to EMBRA3000. A similar procedure has been adopted for a mining a first set of 18,510 BAC-end sequences when 2,406 microsatellites were detected and 1,896 primer pairs designed. This work is rapidly expanding the number of markers beyond the ~300 originally developed from enriched libraries and currently mapped on a reference map. Three important aspects must be pointed out in this respect: (1) EST-derived microsatellites efficiently complement the ones we developed from enriched genomic libraries sampling different portions of the *Eucalyptus* genome (sequence comparison among EST derived microsatellites and genomic derived ones did not result in any redundant markers); (2) microsatellites into transcribed regions should be evolutionarily older than those in noncoding regions and thus

are expected to be more polymorphic; (3) genetic mapping of EST-derived microsatellites enriches the map with transcriptional information opening up the perspective of co-localization of QTLs and candidate genes in regions of higher recombination.

Financial support: Genolyptus project (Ministry of Science and Technology and group of supporting forest based companies).

S5.34p

MICROSATELLITE BASED QTL MAPPING AND VALIDATION ACROSS MULTIPLE PEDIGREES OF *EUCALYPTUS*

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The construction of genetic maps and detection of QTL (Quantitative Trait Loci) for economically relevant traits has been accomplished in *Eucalyptus* mainly with dominant RAPD and AFLP markers. These markers have limited information content and are almost useless for comparative mapping studies and QTL validation across unrelated maps and pedigrees. Microsatellite markers, on the other hand can easily be transferred not only across individual trees but also *Eucalyptus* species. The availability of transportable, multiallelic microsatellites represents a fundamental tool to carry out linkage mapping, QTL validation and effectively move from phenotypes to genomic regions controlling traits of interest for marker-assisted selection. This work consolidates the construction of multiple genetic maps based exclusively on microsatellites and the detection of QTLs for several wood properties. It shows the possibility of performing comparative map analysis between independent experiments, specifically involving QTL location. Genetic maps were constructed for three independent genetically unrelated families using in each one a set of 188 F1 individuals. The first involved a cross between two elite Rio Claro natural hybrid trees involving predominantly *E. grandis* and *E. urophylla*. The other two maps were derived from crosses between pure *E. grandis* and *E. urophylla* select trees and the F1 progeny was cloned and planted in replicated trials in five environments throughout Brazil. A variable number of microsatellite markers between 120 and 180 were genotyped in multiplex systems with fluorescent automated detection and mapped on 11 linkage groups. Individual genetic maps were built for each parent using Mapmaker/EXP and integrated maps with JoinMap and Outmap. Map construction used as reference the integrated map involving 234 markers developed earlier by Brondani et al. (2005, submitted) and added new markers developed from genomic shotgun as well as EST sequences. The integrated maps typically covered between 80 and 95% of the estimated recombination-based genome size. QTLs detection was accomplished under different models with Mapmaker/QTL, QTL Cartographer and the in-house developed Tree-QTL program. In the cross involving hybrid parents, 10 QTLs were detected for parent clone 235, with LODs varying between 2,9 (lignin content) to 4,2 (specific wood consumption). For hybrid parent 221, five QTLs were detected with LODs varying from 2,9 (cellulose yield) to 4,8 (basic wood density). Several QTLs were also found in the other two pedigrees. Comparative QTL mapping across the three pedigrees as well as to QTLs and candidate genes mapping carried out in *E.globulus* by other research groups, revealed a number of syntenic QTLs for cellulose yield, lignin content and for different but correlated fiber traits as well as candidate genes for the lignification pathway. These are novel and exciting results for *Eucalyptus* as they reveal the first QTL validation data across totally

unrelated pedigrees and demonstrate the power of using higher density of microsatellites for QTL validation, thus allowing precise determination of target genomic regions for upcoming association mapping studies, high resolution mapping and eventually marker assisted selection.

Financial support: Genolyptus project (Ministry of Science and Technology and group of supporting forest based companies).

S5.35p

DEVELOPMENT OF MARKER ASSISTED SELECTION TECHNOLOGY FOR APPLE AND PEAR BREEDING IN SOUTH AFRICA

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The development of new cultivars is important for the future competitiveness of the South African deciduous fruit industry. Previously, breeding has used traditional approaches with selection based on fruit quality only. However, in the last decade the approach has changed to incorporate the quantitative analysis of traits of importance to the producer, including the selection for disease resistance at early stages of the breeding program. The recent development of a framework genetic map for apples, together with the release of EST data, provides the basis for the development of new genetic markers that can be used to create higher resolution genetic maps for QTL mapping and marker assisted breeding. At present the total number of markers in development and multiplex analysis is approximately 600, which will provide the basis for a portable framework map for a wide range of genetic mapping projects in apple and pear. The genetic mapping projects in progress cover traits such as disease resistance (apple scab, powdery mildew, woolly apple aphid), dormancy and bud-break, and fruit quality, using populations of seedlings or mature trees as appropriate. These will be used to identify markers linked to QTL, which can then be used in marker assisted selection in the breeding program. We describe the development of a high throughput marker assisted selection system, in order to achieve the selection for multiple markers linked to the desired traits in a single analysis. This involves the use of automated and semi-automated steps to ensure the integrity of sample identity, and the multiplexing of reactions, in order to achieve high throughput, low cost analysis.

S6. Molecular biology and biotechnology of tree development

S6.1

GENETIC MECHANISMS REGULATING THE VASCULAR CAMBIUM AND SECONDARY GROWTH

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Evidence from both microarray analysis and developmental genetic studies indicate significant overlap in the genes and mechanisms regulating the shoot apical meristem and the vascular cambium. This has important implications for developing strategies to define the genetic mechanisms regulating secondary growth, and the evolutionary processes leading to woody growth. To further define the overlapping mechanisms regulating the shoot apical meristem and vascular cambium, we have cloned and characterized the *Populus* orthologs of two key regulators of the shoot apical meristem, SHOOTMERISTEMLESS (STM) and BREVIPEDICELLUS (BP, also known as KNAT1). The *Populus* orthologs of STM and BP are expressed in both the shoot apical meristem and the cambium region, and misexpression of these genes results in phenotypes with defects in secondary growth and wood formation. Microarray analysis of plants overexpressing either the *Populus* STM or BP orthologs reveals a complex role for these genes, including regulating genes involved in secondary cell wall synthesis and lignification, and interaction with pathways involving traditional plant growth regulators. Biochemical analysis of cell wall components confirm changes in cell wall synthesis and lignification indicated by microarray analysis.

S6.2

ANALYSIS OF THE REGULATION OF CAMBIAL MERISTEM ACTIVITY USING A GENOMICS APPROACH

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Plant meristems cycle between active and dormant states. The correct temporal synchronization of active and dormant states with seasonal changes is critical for the survival of the perennial plants. For example, in trees of boreal forest, inability to terminate growth and acquisition of cold hardiness prior to the onset of winter leads to irreversible damage to the meristem cells. The goal of our research is to elucidate how environmental and hormonal signals regulate the induction of dormancy and cold hardiness at the molecular level. Using a combination of genetics and genomics approaches we are identifying transcriptional and metabolic programs associated with the induction and maintenance of dormancy as well as cold hardiness in the model tree poplar. This data is being used to identify key regulators of dormancy and cold hardiness and characterize the role of selected candidate genes by generating transgenic plants with altered expression of these genes.

S6.3

THE DORMANCY TRANSCRIPTOME IN APICAL BUDS OF POPLAR

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The seasonal cycle of growth and dormancy is a distinct character of perennial plants. The transitions of meristems into and out of dormancy are of fundamental importance to plant productivity and survival of adverse environmental conditions. Irrespective of its great importance, the process of bud development and dormancy is poorly described at the molecular level. We conducted cDNA-AFLP and microarray experiments (the latter in collaboration with UPSC) to describe the transcriptome during dormancy induction, dormancy and dormancy release in apical buds of poplar. Furthermore, transgenic poplar up- or down regulating the ABI3 gene, encoding a transcription factor that is necessary for correct bud set in autumn (Rohde et al., 2002), were included to gain a deeper insight into the formation of the bud structure. The expression profiles of 162 genes from the cDNA-AFLP and 1237 genes from the 25K poplar microarray are integrated to map and describe the molecular processes during dormancy. A large overlap with genes expressed during cambial dormancy (Schrader et al., 2004) denotes a set of genes commonly related to dormancy establishment in the various meristems.

Rohde, A., Prinsen, E., De Rycke, R., Engler, G., Van Montagu, M., Boerjan, W. PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. Plant Cell 14, 1885-1901 (2002)
Schrader, J., Moyle, R., Bhalerao, R., Hertzberg, M., Lundeberg, J., Nilsson, P., and Bhalerao, R.P. Cambial meristem dormancy in trees involves extensive remodelling of the transcriptome. Plant J. 40, 173-187 (2004)

S6.4

DIURNAL RHYTHMS OF TRANSCRIPTOME AND METABOLOMES DURING PHOTOPERIODIC REGULATION OF SHOOT GROWTH CESSATION IN *POPULUS*

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Photoperiodism or day length sensing is one of the most important environmental cues for plants. By detecting changes in day length a plant can adapt to seasonal changes and e.g. recognise the correct time for flowering and entering dormancy. We have used hybrid aspen (*Populus tremula* X *P. tremuloides*) to study short-day induction of dormancy. A photoperiodic experiment was conducted, where we collected *Populus tremula* X *P. tremuloides* leaves exposed to different day lengths under a 96 hour period. The gene expression was analysed with cDNA microarrays and their metabolite profiles patterns were analysed with GC-MS. These methods generated large and complex datasets that were analysed with different methods such as Principal Component Analysis (PCA) and Partial Least Squares (PLS). We have combined the information from the gene expression and the metabolomic study to find how certain genes covariates with their metabolomic counterparts, and which genes and metabolites, respectively that have unique expression profiles.

S6.5

REGULATION OF FLOWERING TIME IN TREES

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Many aspects of the regulation of flowering differ between long-lived perennial plants, like forest trees, and the well studied annual plant *Arabidopsis thaliana*. We are studying the functional conservation of a genetic pathway known to regulate *Arabidopsis* flowering in poplar trees. By using Poplar homologues of genes regulating *Arabidopsis* flowering time, we can induce extremely early flowering in transgenic poplars with the production of normal inflorescences. These studies suggest mechanisms for how the juvenility-to-maturity transition is regulated in trees, and how the tree can cycle between phases of vegetative and reproductive growth. Furthermore, our data suggests that poplar flowering and the short-day induced but set in the fall is regulated by the same factors and provides an interesting connection to the regulation of these processes by gibberellins.

S6.6

TRANSCRIPTIONAL CONTROL OF CELL IDENTITY AND DIFFERENTIATION IN THE VASCULAR CAMBIUM IN RELATION TO SEASONAL CHANGES AND GROWTH ACTIVITY

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Plant growth is the result of cell proliferation in meristems, which requires a careful balance between the formation of new tissue and the maintenance of a set of undifferentiated stem cells. Studies have provided important information on several genetic networks responsible for stem cell maintenance and regulation of cell differentiation in the apical meristems of shoots and roots. Until recently, however, very little has been known about the regulatory mechanisms underlying the growth and maintenance of the secondary meristem of trees, namely the vascular cambium. Through high-resolution transcript profiles, substantial differences of the transcriptome was discovered within the cell proliferating zone of the cambium and the differentiating zones of xylem-and phloem mother cells, at the time of fully active growth (Schrader J. et al. Plant Cell. 2004 Sep;16(9): 2278-92). Here we present new expression data from an extended study of high-resolution transcript profiles within the wood forming zone we have studied high-resolution transcript profiles over the cell proliferation- and maturation zones of the cambium, from the state of inactive growth at dormancy in early spring, until fully active and expanded cambium. We provide data that not only covers transcriptional changes and modulations of the vascular cambium at single state of growth but through out the whole first dynamic highly regulated growth period. We thus capture global transcriptional differences and regulations of the transcriptome to detect fine tuned modifications of potential key regulators of the cambium in correlation to the seasonal changes and growth activity during this period.

S6.7

MASTER REGULATORS OF PROTOXYLEM AND METAXYLEM VESSEL FORMATION

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The zinnia *in vitro* vessel differentiation system in which isolated mesophyll cells are induced to differentiate into tracheary elements, components of vessels, has successfully been used for analyzing molecular bases underlying the vessel formation. To elucidate the gene regulatory mechanisms, we carried out cDNA microarray analysis in the zinnia system. Also, we recently established the *in vitro* vessel differentiation system from subcultured *Arabidopsis* cells and carried out the GeneChip analysis. Such analyses identified many genes up- or down-regulated significantly during the differentiation. Among them, we focused on zinnia *Z567* and its *Arabidopsis* homologues, *VASCULAR-RELATED NAC-DOMAIN1* (*VND1*) to *VND7*, encoding NAC-domain proteins. Promoter analysis revealed that the *VND1* to *VND7* were expressed in vascular cells preferentially. Surprisingly, overexpression of *VND6* and *VND7* can induce transdifferentiation of various cells into metaxylem- and protoxylem-like vessel elements, respectively, in *Arabidopsis* and poplar. A dominant repression of *VND6* and *VND7* specifically inhibits metaxylem and protoxylem vessel formation in roots, respectively. These findings suggest that these genes are master regulators of metaxylem and protoxylem vessel formation.

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Genes & Development, 19: 1855-1860, 2005

S6.8

UNDERSTANDING CAMBIAL DEVELOPMENT AND WOOD FORMATION IS A PATCHY BUSINESS

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The analysis of somatic tissue sectors has greatly aided our understanding of fundamental issues relating to pattern formation in animal and plant tissues. Important discoveries included the existence of parasegments in *Drosophila*, the clonal origin of cell layers in stratified plant shoot apical meristems and the independent developmental control of flower organ initiation and identity in *Arabidopsis*. In order to expand such studies to secondary tissues in trees we created transgenic wood sectors in a variety of tree species. In stems of these trees transgenic tissue patches developed from individually transformed cambial initials and other cells within the cambium/procambium both *in vitro* and *in vivo*. Our earlier studies indicated that such methods are useful for promoter studies and for the analysis of candidate gene and transgene activity during wood formation and provide opportunities for investigations into the molecular basis of cambium differentiation and wood formation. Importantly, however, the size, distribution and delineation of transgenic sectors in their own right hold valuable clues about the controlling processes underlying meristem differentiation. Careful analysis of transformed sectors and their boundaries is now being used to study in detail the developmental fate of cambial initials and deduce rules of morphogenetic pattern formation during stem development and secondary growth processes. First results of our current and ongoing investigations will be presented.

S6.9

CHARACTERIZATION OF DEVELOPMENTAL PROGRAMMED CELL DEATH IN XYLEM FIBERS OF POPLAR (*POPULUS TREMULA X TREMULOIDES*): FROM ANATOMICAL TO MOLECULAR INSIGHTS INTO THE DEATH PROCESS OF WOOD

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Tree development is contingent, among other processes, on programmed cell death (PCD) occurring throughout life time in a wide range of tissues, from seed to leaf, including wood fiber. Wood development ends up with death of fibers on a programmed way giving the wood all its characteristics but underlying PCD mechanisms are lacking attention. Xylem wood fibers evolve from the cambial region towards death, at which time they become empty and serve as support for the tree when growing higher by means of secondary cell wall, i.e. lignins, starting depositing early previous to fiber death, correlating lignin deposition and death of xylem fibers. In our study, confocal microscopy optimized for quenching lignin autofluorescence revealed structural changes in nucleus throughout wood fiber development, from DNA condensation to its fragmentation supported by TUNEL staining. These morphological changes are associated to the rapid vacuolar collapse. In addition the role of autophagy was unfolded for fiber survival in early xylem tissue as well as for degradation of fiber contents in the death region, as visualized by high pressure-freezing microscopy. From the 25k poplar cDNA microarray on wood sections, gene ontology analysis unravelled increased gene expression in reorganization then in disassembly of the cytoskeleton beside a final increase in proteolysis genes over the wide fiber cell death region. Interestingly as well, most of the genes related to auxin response were upregulated in this region. Thoroughgoing microarray analysis disclosed 15 genes with expression patterns specific to the fiber cell death region. More precisely a *Vacuolar Processing Enzyme (VPE)* and a *metacaspase* genes were found specifically upregulated before xylem fibers death. The *In-Situ* RT-PCR over these genes confirmed their co localization specifically to fibers. VPE expresses in vacuoles. The metacaspase gene was found associated to mitochondria, linking the role of mitochondrial burst to fiber death. Finally we revealed the importance of some additional genes specifically upregulated in the fiber cell death region of poplar, such as a *major-intrinsic protein* , an *ACL5* and a *c-peptidase* genes, by looking at *Arabidopsis* transgenics showing defects in xylem cell formation and death. In our results we describe fiber cell death on the anatomical level and support the role of autophagy for fiber content degradation. We reveal as well that a β-VPE, a metacaspase and a major intrinsic protein, candidate genes, might act in the pathway toward death of xylem fibers and probably might participate in the triggering of the vacuole collapse, as well as mitochondrial disruption. We emphasize on the role of metacaspase and VPE genes as major components of cell death in our system and we offer a comprehensible understanding of PCD occurring in xylem fibers.

S6.10

GENOMICS OF CONIFER EMBRYO DEVELOPMENT

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The development of an embryo inside a seed recruits many different biochemical pathways to support cell division and differentiation in a timely manor. Regulatory mechanisms that direct embryo development have been suggested by presence of metabolites, and differential expression of genes coding for specific pathway components. Somatic embryos appear to follow the same developmental paths as the zygotic embryos based on morphology and molecular evidence. Currently available culture protocols is however not optimal for all genotypes. We seek to increase our understanding of the development of the zygotic embryo to be able to improve growth and yield of somatic embryos for clonal propagation. A Georgia Tech-TIGR project has identified and catalogued genes that are expressed in pine tissues during all stages of embryo development through generation of single-pass sequence reads from the 5' end of randomly selected cDNA clones from pine embryo cDNA libraries. To date approximately 69,000 cDNA sequences have been generated from which 12,237 contigs and singletons have been identified. A loblolly pine EST database (Pine Gene Index) has been constructed using publicly available sequences and ESTs generated in the course of this project (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=pinus). cDNA arrays using approximately 10,000 clones from our project are currently being made. By microarray gene expression analysis, describing global patterns of expression, and analyses of specific developmental genes known from angiosperm embryo development, we are gaining information on specific developmental events of importance for the "natural" development of the embryos.

S6.11p

SPACE INVADERS: NOT ALWAYS THE BAD GUYS

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Vessel-associated ray cells in the sapwood/heartwood transition zone of some angiosperm tree species form tyloses during a final differentiation process that may occur more than five years after their original differentiation from ray initials in the cambium. While there is some consensus as to the biophysical basis of tylosis initiation the molecular basis of their expansion is unknown. Extracting sufficient RNA from differentiating transition zone ray cells for cDNA construction and gene discovery is compromised by asynchronous cellular activity and interference from polyphenols dispersed throughout the xylem. To circumvent these problems we used massive wound response, induced by crown removal, to trigger extensive and synchronised tylosis formation in outer sapwood of stems of 10-year-old *Eucalyptus nitens* trees. Earlywood containing tyloses was carefully chipped from discs cut from stems and pulverised to a fine powder for RNA extraction and cDNA preparation. We targeted expansin genes as candidates for tylosis expansion and sequencing of PCR products revealed sequences with strong homology to known alpha-expansin cDNAs. Ray cells differentiating to tyloses were the only cells expanding in the sample tissue providing strong circumstantial evidence that the alpha-expansin gene detected is in fact directly associated with tylosis expansion. Here we present our gene discovery strategy and further downstream characterisation of candidate genes involved in tylosis formation.

S6.12p

DEVELOPMENTAL ANALYSIS OF THE TRANSITION FROM PRIMARY TO SECONDARY GROWTH IN POPLAR

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Vascular secondary growth results from the activity of the vascular cambium, which produces secondary phloem and secondary xylem. By means of cDNA-AFLP analysis along *Populus tremula x P. alba* (clone INRA 717-1B4) stems, several potential regulatory genes involved in the progressive transition from primary to secondary growth were identified. A total of 83 unique transcript-derived fragments (TDFs) were found to be differential between the top and the bottom and the stem. An independent RT-PCR expression analysis validated the cDNA-AFLP profiles for 19 of the TDFs. Among these, six correspond to new genes encoding putative regulatory proteins. Emphasis was laid upon two genes encoding respectively an AP2/ERF-like transcription factor (PtaERF1) and a RING-H2 finger protein (PtaRHE1), which differential expression was further confirmed by reverse northern analysis. *In situ* RT-PCR revealed that *PtaERF1* was expressed in phloem tissue and that *PtaRHE1* had a pronounced expression in ray initials and their derivatives within the cambial zone. Expression analysis revealed that *PtaERF1* is induced by wounding and *PtaRHE1* is induced upon ABA exposure. To further characterize *PtaERF1*, transgenic lines overexpressing this gene were established. Phenotypical analysis showed that T2 homozygous lines had longer stems with longer internodes and exhibited a precocious transition from vegetative to reproductive stages. For *PtaRHE1*, promoter-driven *GUS* expression was studied for *ATL2*, the closest *Arabidopsis* homolog to *PtaRHE1*. In mature plants, a strong GUS signal was detected in the part of the stem where secondary growth takes place. These results suggest a potential role of these genes in vascular tissue development and/or functioning.

S6.13p

CDNA MICROARRAY GENE EXPRESSION ANALYSIS OF THE TRANSITION FROM JUVENILITY TO MATURITY IN POPLAR

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One of the most fascinating differences between herbaceous species and trees is the enormous time difference in the transition from juvenility to maturity. By the use of microarray analysis we have studied the genes involved in this transition, focusing on similarities and differences between the annual plant *Arabidopsis* and the perennial tree Poplar. For the transition experiments we used an age series of a Poplar clone, which had been planted out continuously in the field. At the time of the experiment, it was at the age of 1, 2,..., 6 years. The Poplar clone initiates flowering between the ages of 4 to 6 years. Samples for the microarray experiments were collected at two separate time points; May and June. We have shown that the Poplar homolog to the *Arabidopsis* gene, *FT*, is very important in regulating flowering in Poplar. By comparing downstream targets of *FT* in *Arabidopsis* and Poplar to the genes which are differentially expressed during the juvenility to maturity transition, we expect to locate important candidate genes for this transition in trees. We have also studied the difference in gene expression on mature and juvenile branches on a reproducing mature tree. Since many trees appear to have an internal gradient between juvenility and maturity with respect to the physiological aspect of the tree, we wanted to see if this internal gradient between juvenility and maturity was mirrored in the global gene expression pattern in different parts of an individual tree.

S6.14p

ETHYLENE AND WOOD FORMATION

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Wood formation is responsive to environmental and downstream physiological conditions. The developmental gradient spanning the cambium, expansion zone and secondary cell wall thickening zone is responsible for determining cell form and fate, and thus wood quality. Ethylene has been associated with a number of aspects of plant development, however only recently has there been any evidence to suggest that it is a candidate plant growth regulator involved in wood formation, in particular tension wood formation. Tension wood is an aberrant woody tissue most obviously characterized by a gelatinous cell wall layer and eccentric growth on the upper side of a leaning angiosperm stem. The dominant negative mutant *etr1-1*, identified in a triple response screen in *Arabidopsis*, has been used to generate lines of ethylene insensitive *Populus tremula x tremuloides* and *Betula pendula*. Treatment of seedlings with 1-aminocyclopropane-1-carboxylic acid, the immediate precursor to ethylene, enhanced radial growth, altered vessel formation and reduced stem height in wild type, but had no effect in the insensitive lines. In the ethylene insensitive Birches, we have observed a significantly lower degree of leaning induced eccentric growth compared to wild type. Despite this, the gelatinous layer was formed in both *etr1-1* transgenic lines and wild type. These results suggest ethylene is involved in a woody growth response.

S6.15p

CLIMATIC ADAPTATION IN *PICEA ABIES* PROGENIES IS AFFECTED BY THE TEMPERATURE DURING ZYGOTIC EMBRYOGENESIS: AN EPIGENTIC MEMORY?

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The temperature during maternal reproduction affects adaptive traits in progenies of Norway spruce (*Picea abies* (L.) Karst.). Seed production in a cold environment advances bud set and cold acclimation in the autumn and dehardening and flushing in spring, whereas a warm reproductive environment delays timing of these traits. We repeated crosses between the same parents and produced seeds under contrasting temperatures. Elevated temperatures were applied at different time points from female meiosis to embryogenesis, followed by full-sib progeny tests in common environments. We measured timing of terminal bud formation, cold acclimation in the autumn and transcription levels of conifer phytochromes PhyO, PhyN, PhyP, and the glycohydrolase PaChi4 in these tests. No progeny differences were found that could be related to temperature differences during pre-zygotic stages and fertilization. In contrast, progeny performance was strongly associated with the degree-days from proembryo to mature seeds. Progenies with a warm embryonic history formed terminal buds later, were less hardy, had higher methylation levels and expressed lower transcription levels of the genes. We hypothesize that temperature during zygotic embryogenesis and seed maturation regulates an "epigenetic memory" in the progeny, possibly involving methylation, epialleles and thus differential expression of genes that may regulate bud phenology, cold acclimation and embryogenesis in Norway spruce.

S6.16p

DELINEATION OF THE LIGNIN BIOSYNTHETIC PATHWAY IN NORWAY SPRUCE USING EST SEQUENCING AND REAL-TIME PCR

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To compare the lignin biosynthetic processes in developing wood of Norway spruce (*Picea abies*) and in a lignin-forming suspension culture model system, 4000 ESTs were sequenced from both tissues. Lignification-related gene expression was high, as 4% and 2% of ESTs, respectively, were potentially related to lignin biosynthesis. For each gene, several unigenes (gene family members) were found. Intriguingly, only one unigene for each gene family contained ESTs from both the tissue culture and wood. A more detailed expression analysis of these genes in several tissues was performed by real-time reverse transcription PCR. Only one unigene of each monolignol biosynthetic gene was highly expressed in developing wood. The same unigene was highly expressed in compression wood also; however, induction of a second gene family member was also seen for PAL, C4H and COMT. CCoAOMT was an exception, as all three unigenes identified were expressed at very high level in both woody tissues. In the tissue culture, expression of monolignol biosynthetic genes was lower as compared to wood, but the same unigene, except for CCR and CAD, was highly expressed in this system also. In needles, roots and phloem expression levels were much lower, but a similar trend in unigene expression was seen. With peroxidases and laccases responsible for lignin polymerisation, the picture was less clear, as different unigenes were expressed in normal and compression wood, and in the tissue culture. In conclusion, our results pinpoint the most likely gene family members responsible for monolignol biosynthesis in Norway spruce. Existence of a common monolignol biosynthetic pathway in normal and compression wood was also supported. The lignifying tissue culture has in common at least the early steps of lignin biosynthesis, although the last reductions might be catalysed by different enzyme isoforms.

S6.17p

DEVELOPMENTAL AND ENVIRONMENTAL EFFECTS IN WOOD FORMATION ON TRANSCRIPTS' ACCUMULATION IN MARITIME PINE

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Wood (secondary xylem) provides both mechanical strength and long-distance transport for water, nutrients and hormones. Despite the simplicity of secondary xylem structure (basically tracheids and rays), conifers' wood is a highly variable material in terms of anatomical, chemical and technological characteristics. Taking advantage of the formidable plasticity in wood characteristics (e.g. resulting from environmental, ontological and genetic factors), we hypothesized that differentially expressed genes/proteins between these different types of wood could correspond to key molecular players controlling the phenotypic variation. To test this hypothesis, differentiating xylem of maritime pine (*P. pinaster* Ait.) was collected along a cambial age gradient (samples taken every 3 years' internodes from top to base on a 30-years old adult tree) and along the growing season (samples taken from beginning of April to end of July). A genomic approach combining large-scale EST, protein sequencing, expression profiling (high density filters macroarray), and statistical analyses, was undertaken to identify the candidate genes for wood properties showing differential accumulation of transcripts and proteins. Different and contrasting expressional patterns were obtained for these candidate genes. Twelve genes differentially expressed in both kinetics were selected and their expressional profiles are currently validated by qRT-PCR on replicated biological samples. Meanwhile, functional validation of some of these genes is ongoing using association studies. This work provides us with new insights into the molecular basis of wood characteristics.

S6.18p

CORRELATION BETWEEN HIGH RESOLUTION ANALYSIS OF AUXIN LEVELS AND EXPRESSION OF AUXIN REGULATED GENES IN THE VASCULAR CAMBIUM OF POPLAR

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Auxin is possibly the most extensively studied of the plant hormones. The effects of auxin are versatile and vary depending on tissue, cell type and developmental stage of the plant as well as interplay with a variety of endogenous and exogenous signals. At a cellular level the effect of the hormone is highly dependent on concentration, which in turn is controlled by synthesis and transport. The major active auxin is Indole-3-acetic acid or IAA. In the wood forming tissues of hybrid aspen (*Populus tremula x tremuloides*) IAA levels form a steep concentration gradient across the developing tissues with a concentration peak in the cambial region. The IAA gradient has been measured with high resolution by measuring hormone levels in tangential cryosections covering the vascular cambium (1). Here we use these results combined with a high resolution transcriptional map over the vascular cambium (2) to show that no known auxin responsive genes have a transcriptional pattern that follow the auxin gradient. The expression pattern of auxin transporters belonging to the Aux1 and PIN families show a similar shape, but the expression peak is displaced compared to the peak of the auxin gradient. We have also performed large scale auxin induction experiments using cDNA microarrays to identify the majority of auxin regulated genes in young leaf and stem tissue of hybrid aspen. Again we find that, despite all the previous knowledge, a majority of all auxin regulated genes identified in our study are not following the auxin gradient. Observing discrepancies between known auxin induced genes and actual auxin levels with cell specific resolution we postulate that the regulation of this hormone is strictly tissue dependent. Based on our results, we question the use of the IAA driven expression of DR5::GUS as a reliable measurement of auxin levels.

1. Tuominen H, Puech L, Fink S, Sundberg B. (1997) A Radial Concentration Gradient of Indole-3-Acetic Acid Is Related to Secondary Xylem Development in Hybrid Aspen. Plant Physiol. 115:577-585
2. Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G. (2004) A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. Plant Cell 16:2278-92

S6.19p

DISSECTING THE INTERACTIONS BETWEEN LIGHT AND AUXIN SIGNALING PATHWAYS IN THE CONTROL OF ADVENTITIOUS ROOT FORMATION

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Adventitious rooting is an essential step in the vegetative propagation of economically important horticultural and woody species. A lack of competence to form adventitious roots by cuttings or explants in vitro occurs routinely and is an obstacle for the clonal propagation and rapid fixation of elite genotypes. Adventitious rooting is known to be a quantitative genetic trait. We have recently characterized mutants of the model plant *Arabidopsis thaliana* affected in their ability to develop adventitious roots in order to identify associated molecular markers that could be used to select genotypes for their rooting ability and/or to get further insights in the molecular mechanisms controlling adventitious rooting[1,2]. We could show that light an auxin may act antagonistically in the regulation of adventitious rooting and could identify through a proteomic analysis of the different genotypes 13 proteins correlating with the adventitious root formation. We also showed that the *Auxin Response Factor 17* (*ARF17*) negatively regulates the expression of auxin inducible *GH3-like* genes and adventitious rooting. Our results strongly suggest that these genes might be major regulators of adventitious root formation. In order to get further insights in the respective role of light and auxin we are now further analyzing the competence to form adventitious roots in mutants affected in the expression of *GH3-like* genes, *ARF10* and *ARF16* that are phylogenetically related to *ARF17* as well as mutants affected in different phytochrome signal transduction pathways.

1 - Sorin et al. (2005) Auxin and Light Control of Adventitious Rooting in *Arabidopsis* Require ARGONAUTE1. Plant Cell 17:1343-1359
2 - Sorin et al. (2005) Proteomic analysis of different mutant genotypes of *Arabidopsis thaliana* led to the identification of thirteen proteins correlating with adventitious root development. (Submitted)

S6.20p

FUNCTIONAL CHARACTERIZATION OF PHLOEM GENES: THEIR ROLE IN PHLOEM DIFFERENTIATION AND FUNCTIONING IN POPLAR AND *ARABIDOPSIS*

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Plant vascular tissues are composed of two basic units, xylem and phloem. Xylem transports and stores nutrients and water, some hormones and provide mechanical support. Phloem distributes photosynthetic products from the leaves to the meristems and other sink organs, but also transports hormones and macromolecules such as proteins and RNAs. Phloem is a way for the propagation of pathogens such as viruses, as well as the target of piercing-sucking insects feeding on elaborate sap. In most woody plants, phloem is also the first tissue produced by the cambium meristem when the vegetative activity starts after wintertime. Despite the key role of phloem in plant development and adaptation to environment, mechanisms controlling its differentiation and functioning are still poorly understood. In order to shed light on these mechanisms, we have undertaken a systematic functional analysis of genes highly expressed in the phloem initials through an integrative approach combining modern genetics, molecular biology, cell biology and biochemistry. A transcriptional analysis of the wood-forming region of poplar was performed at the UPSC[a,b] and genes highly expressed in the phloem, cambium and xylem regions were identified. *Arabidopsis thaliana* horthologues for phloem expressed genes are found and T-DNA insertion lines are currently being analyzed. Production of RNAi mutants will be undertaken in poplar. Protein fusion with either GFP or GUS will be made to confirm the expression pattern. In this poster we will present the first results obtained through this systematic analysis.

a – Hertzberg *et al.,* (2001) A transcriptional roadmap to wood formation *Proc Natl Acad Sci USA* 98: 14732-7
b – Schrader *et al.,* (2004) A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. *Plant Cell* 16(9): 2278-92

S6.21p

PROTEIN EXPRESSION DURING WOOD FORMATION

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Wood is the result of a process of cellular differentiation, which involves the biochemical events of cell division, elongation and radial expansion, cell wall thickening, and autolysis. The understanding of the physiological and molecular mechanisms involved in wood formation remains on its infancy. Only a few studies carried out at the transcriptome level have provided initial insights into the players involved in this biological process. The aim of this work was to describe the main proteins expressed in wood forming tissue (differentiating secondary xylem) using 2-DE and mass spectrometry. Maritime pine (*Pinus pinaster* Ait.), a conifer of major economical and ecological importance in South-western Europe was used as a model forest tree species. In order to take into account the natural variability generally found in conifer wood in terms of anatomical characteristics, chemical composition and mechanical properties, a reference map was established by combining individual maps of the six types of wood usually found in an adult tree: i.e. early wood and late wood (respectively formed at the beginning and the end of the growing season), juvenile wood and mature wood (respectively formed at the top and at the base of a tree), compression wood and opposite wood (respectively formed at the lower and upper side of a leaning stem). Using high resolution 2-DE with linear pH gradient ranging from 4 to 7, a total of 1039 spots were detected. Out of the 240 spots analyzed by LC ESI-MS/MS, 172 (71.7%) were identified, while 31 (12.9%) presented no homology in the databases, and 37 (15.4%) corresponded to mixtures of proteins. Out of the 57 spots analyzed by MALDI-TOF MS, only 9 (15.8%) were identified 6 being also identified by tandem MS. The function of 9 other proteins was established by internal microsequencing. Over the 184 identified proteins, most (87%) play a role in defence (20.1%), carbohydrates (16.3%,) and amino acid (14.7%) metabolism, genes and proteins expression (13.6%), cytoskeleton (7.6%), cell wall biosynthesis (5.4%), secondary (4.9%) and primary (4.4%) metabolisms. A summary of the identified proteins and their putative functions is presented. This information was introduced into the PROTICdb database and is accessible at http://cbi.labri.fr/outils/protic/ProticDB.php. For 95 functions, the average protein amount was compared with their respective transcript abundance as quantified through EST counting in a composite cDNA-library constructed with mRNAs extracted from wood forming tissue. Finally, expression level of the identified proteins was compared between the six types of wood.

S6.22p

IDENTIFICATION OF CANDIDATE GENES CONFERRING SPECIFIC PROPERTIES TO DISTINCT TYPES OF *PINUS PINASTER* AIT. WOOD

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Genomic technologies have been used as a powerful tool to identify genes involved in different biological processes with interest for agriculture and forestry. Our goal is to apply these new technologies to identify genes involved in processes with much interest for wood and paper industries. We are investigating molecular aspects of wood formation in maritime pine (*Pinus pinaster*, Ait.) as a model of southern Europe conifer. The strategy we are following is to detect genes which are exclusively expressed or overexpressed in differentiating xylems which develop any of the following types of wood in maritime pine trees: juvenile, mature, compression or opposite wood. A screening procedure have been carried out involving: 1) enrichment in differentially expressed genes by constructing SSH libraries, 2) screening 4000 subtracted cDNA clones by microarray analyses, 3) validation of the differential expression feature by northern blot analysis with trees from two populations. We have identified sets of genes showing particular patterns of expression, suggesting that they can be candidate genes conferring specific properties to one o more type of wood.

S6.23p

ISOLATION AND CHARACTERIZATION OF CELLULOSE SYNTHASE GENES FROM SPRUCE

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The modulation of cellulose biosynthesis directly influences many aspects of plant growth and development including, but not limited to, cell division and expansion, plant morphogenesis, response to environmental cues, and ultimately the structure of the cell wall. A thorough understanding of the intricacies of the biosynthetic processes governing the deposition of cellulose throughout the life cycle of plants is, therefore, pivotal to understanding cell wall ultrastructure and chemistry. A significant amount of research has recently revealed that the genes directly involved in cellulose production, cellulose synthases (*CesA*) and the putative cellulose synthase-like (*Csl*) genes, are part of large multi-gene families. The recent completion of the poplar genome has provided an unprecedented opportunity to decipher the enigmatic processes of cellulose biosynthesis in trees, and can complement the existing information generated from the *Arabidopsis* genome. However, similar knowledge has yet to be acquired from other trees, including the gymnosperms, such as spruce. The goal of this research was to isolate the *CesA* multi-gene family involved in secondary cell wall formation from spruce (*Picea glauca*). Using genetic information compiled from a variety of plants, a spruce EST library was screened for putative *CesA* sequences. Employing partial *CesA* sequences and RACE PCR techniques, full-length *CesA* cDNAs have been isolated and characterized. The identified *CesA* cDNA sequences are being compared to known *CesA* and *Csl* sequences from a variety of terrestrial plants. In addition, the sequences of the hypervariable II domains (HVRII) will be examined to understand the phylogeny of spruce *CesA* proteins and their relationship with other trees, both deciduous and gymnosperms. The functionality of the putative secondary cell wall *CesA* genes isolated and cloned from spruce is being investigated using complementation studies with *Arabidopsis* *CesA*-deficient mutants. This information should further aid in elucidating our understanding of the role of the *CesA* gene family in cellulose production in gymnosperms.

S6.24p

TRANSCRIPTION FACTORS TO CRACK THE LOCK ON THE WOOD TRANSCRIPTOME

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With the development of genomic technologies like microarrays, it is expected that a more complete picture of the transcriptome will emerge, ultimately revealing its overall modulation at different phase of tree growth and development. Our experimental approach brings together microarray transcript profiling and transcriptional regulators believed to control gene expression in key processes of secondary xylem formation, i.e. wood formation, as means to address the underlying mechanism of transcriptome regulation. We identified conifer and poplar genes that are homologous to *Arabidopsis* genes previously linked to vascular development. These include HD-zip, KNOX, MYB, LIM domain, GRAS and other transcription factors. An overview of the molecular characterization and the expression of selected transcription factors will be presented. As a means to further define the role that these genes play, a suite of transgenic poplar and spruce trees has been created that mis-express each of the candidate genes. Early results from gain-of-function and loss-of-function experiments indicate that several of the targeted genes altered vascular development. Detailed analysis of transgenics spruce over-expressing MYB transcription factors has identified two members to this family that putative positive regulators of lignin biosynthesis enzymes. We have begun to investigate the role of the mis-expressed genes in transcriptional control through transcript profiling experiments with custom low redundancy cDNA microarrays both in spruce and in poplar. Results from microarray experiments will be presented.

S6.25p

ELUCIDATING THE GENETIC NATURE OF WOOD QUALITY IN SITKA SPRUCE – FROM MICROARRAY TO FIELD

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The aim of this project is to determine the genetic basis of wood quality traits, particularly wood density. This aim will be achieved by studying differential gene expression between 'high' and 'low quality' trees, defined as those with high and low wood density. This project is in collaboration with the Forestry Commission UK and the Forestry Genome BC project at the University of British Columbia, Vancouver. Very little is understood about the traits affecting wood quality, and no research has yet been undertaken to identify genes important for wood quality in Sitka spruce, an important forestry tree in both the UK and Canada. An experimental site set up by the Forestry Commission in the Scottish Borders contains 750 trees, 50 clones each replicated 15 times. 10 clones were selected based on their density and growth measurements, and all 15 replicates of each of the 10 clones were sampled from (150 trees in total). Xylem was collected from each tree at three points during the growing season (May, July and September). 60 trees were selected for analysis, and RNA from these individuals was hybridised to a 21.8 K spruce array at UBC. The trees selected covered a range of densities. Our latest analysis of these gene expression data will be presented.

S6.26p

ISOLATION AND CHARACTERIZATION OF MIRNAS AND SIRNAS IN DIFFERENTIATING WOODY TISSUES OF *EUCALYPTUS*

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MicroRNAs (miRNAs) and short interfering RNAs (siRNAs) are small (~ 21 to 24 nt) RNA molecules that direct gene silencing by targeting specific mRNAs for either translational repression or direct cleavage. miRNAs have been shown to play specific roles in the regulation of complex developmental pathways in plants and animals. In plants, miRNAs are involved in the regulation of developmental processes such leaf morphogenesis, flowering time, floral organ identity, meristem identity, and apoptosis. Wood formation (xylogenesis) involves differentiation of a lateral meristem tissue (the vascular cambium) into mature xylem cell types, a process that usually ends in programmed cell death. Do miRNAs play a role in the regulation of wood development? We are addressing this question by characterising nuclear encoded miRNAs and endogenously formed siRNAs isolated from three differentiating woody tissues of *Eucalyptus grandis*, namely mature xylem, immature xylem and phloem. These small RNAs are being compared to similar molecules recently isolated from *Populus trichocarpa* in order to determine evolutionary conservation of this regulatory mechanism in these two forest tree genera. We will report on miRNAs that have been identified in *P. trichocarpa* tissue culture plants and mature *Eucalyptus grandis* tissues and which show sequence conservation in *Arabidopsis*, rice and maize, as well as, putative tree-specific miRNAs.

S7. From somatic embryos to genetic engineering

S7.1

AUXIN SIGNALING DURING PATTERN FORMATION OF SOMATIC EMBRYOS OF *LARIX DECIDUA*

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The plant hormone auxin has been implicated in regulating embryonic pattern formation in plants. To study the role of auxin and its transport in embryo development of conifers in the absence of maternal factors, somatic embryogenesis of *Larix decidua* was used as a model system for developmental studies. The addition of auxin transport inhibitors to the culture medium caused alterations of the embryo phenotype. The auxin efflux inhibitor N-naphthylphthalamic acid (NPA) interfered with cotyledon separation or apical-basal axis formation. Similar morphological effects have been provoked by the conjugation of free indole-3-acetic acid (IAA). The soybean GH3 promoter has been shown to be suitable for visualizing auxin distribution *Larix decidua* embryos. Cellular auxin localisation was firstly detected in the late globular stage and revealed a specific distribution pattern with restriction to the differentiating root cap. Exogenous auxin induced a specific expression pattern which changed during embryo development. In the earliest stages a ubiquitous expression in the embryo and suspensor cells was observed, whereas at later stages the expression was localised to the basal pole of the embryo. Treatments of transgenic embryos with auxin transport inhibitors and an antiauxin indicated correlations between auxin distribution and apical-basal patterning. During different developmental stages free IAA concentrations were directly measured using a microscale technique and exhibited a maximum during the late globular stage just before transition to the bilateral symmetry of the embryo. The results indicate evolutionary conserved mechanisms of auxin signalling between conifers and angiosperms during embryonic pattern formation.

S7.2

IDENTIFICATION OF GENES RELATED TO ADVENTITIOUS ROOTING CAPACITY IN PINE AND CHESTNUT

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Maturation-related decline of adventitious root formation is one of the most important barriers to clone selected trees. To identify novel expressional candidate genes involved in the rooting capacity of forest species, three suppression subtractive hybridization (SSH) libraries from pine and chestnut rooting-competent tissues under inductive conditions have been constructed. The analysis of 1,352 ESTs showed that libraries were enriched in clones with putative function in adventitious root formation. A significant number of clones showed identity with genes related to auxin response, cell cycle control, root determination and differentiation, or adventitious regeneration in both species. Large-scale expression profiling and expression of individual genes in tissues with different rooting capacity will help us to select the most appropriate candidate genes, and to gain a better understanding of the mechanisms involved in the expression of maturation-associated traits in gymnosperms and angiosperms. Function of identified genes is being tested in model systems to verify their putative biological role in the rooting capacity.

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S7.3

FIELD PERFORMANCE OF SCOTS PINE CUTTINGS OF FASCICULAR SHOOT ORIGIN ROOTED BY *AGROBACTERIUM*

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Scots pine (*Pinus sylvestris* L.) is one of the key species of forest ecosystems on large areas of boreal forest zone, having a remarkable economic impact. In Nordic countries, Scots pine has been involved in forest tree breeding activities since their beginning in 1940's. The main reason for slow progress in genetic improvement is drawn-out testing based on progeny trials. If vegetative propagation of species was possible, could clonal testing remarkably facilitate breeding. Vegetative propagation of Scots pine has proved difficult, although several techniques, e.g. rooted cuttings, organogenesis, and somatic embryogenesis, have been developed. Up to date, reports on the field performance of vegetatively propagated Scots pine material have not been published. The aim of the present study was to examine the field performance of 10-11 year old Scots pine cuttings of fascicular shoot origin that were rooted either with *Agrobacterium* or IBA hormone treatment. The original rooting success of Scots pine fascicular shoots in the years 1993 and 1994 varied depending on rooting treatment, timing of rooting, and genetic background of cuttings. Examining the field performance of the rooted Scots pine cuttings showed that vegetatively propagated Scots pine material is able not only to survive but also grow well following out planting. There was, however, a lot of variation among the cuttings, i.e. also inferior cuttings characterised by weak growth, poor stem quality, and plagiotropic growth habit. Several factors, including the rooting treatment used, timing of rooting, morphology of cutting root system and genetic background of the cuttings were found to affect their field performance.

S7.4

EFFICIENT TRANSFORMATION METHOD OF *EUCALYPTUS GLOBULUS* AND *EUCALYPTUS CAMALDULENSIS*

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Eucalyptus is very important material for the pulp and paper industry. Genetic engineering is a useful tool for breeding of woody plants because they have a long life span. However, it is difficult to improve the transgenic *Eucalyptus* because of its low transformation frequency. First, we used hypocotyl of *Eucalyptus camaldulensis* and *E. globules* as a material of the *Agrobacterium*-mediated transformation method. We could obtain transgenic *Eucalyptus* by improving the bud regeneration medium, but the transformation frequency was very low. As a result of intensive studies, we found that when a hypocotyl having a shoot meristem was used as an inoculation material, the desired gene was efficiently introduced into a base part of the shoot, and the regeneration frequency from the part was high. Then, we succeeded in the efficient transformation on both *Eucalyptus* species by kanamycin selection (1-5%) and ipt selection (5-10%). In case of *E. globules*, it is a problem that the rooting is difficult. By photoautotrophic culture method fertilized the carbon dioxide, we could obtain transgenic shoot with healthy rooting. Until now, we introduced several useful genes into both *Eucalyptus* species, and confirmed the transgene in most of the kanamycin resistant shoot by southern blot analysis.

S7.5

PPRAB1, A RAB-RELATED SMALL GTP-BINDING PROTEIN IS PRESENT IN *PINUS PINASTER* AND IS EXPRESSED PREDOMINANTLY IN EARLY EMBRYOGENESIS AND SEEDLINGS

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Rab-related small GTP-binding proteins are known to be involved in the regulation of the vesicular transport system in eukaryotic cells. The identification of a Rab coding full-length cDNA *PpRab1* from maritime pine embryogenesis was previously reported (Gonçalves et al., in press). Here we report the characterization of transcript *PpRab1*. Phylogenetic analysis showed that PpRab1 is more closely related to the Rab family and within this, PpRab1 protein was found to cluster with *Arabidopsis* subfamily AtRABE, which members are known to regulate ER-to-Golgi membrane trafficking steps. Expression analysis of *PpRab1* transcript by real-time PCR revealed a peak of expression in stages T1, T2 and T3 stages of zygotic embryo development, and a decreasing expression as embryo matures. The *PpRab1* transcript is not embryo-specific as it was found present in roots, cotyledons and hypocotyls. Transcript *PpRab1* appears to be expressed in a lower level in roots when compared to hypocotyls and cotyledons. An increase of *PpRab1* level of expression is observed when seeds are germinated and collected at different time points of development (G1-G4). Somatic embryos were also tested for the presence of *PpRab1* transcript. The *PpRab1* transcript appears to be present since the early development of somatic embryos and become more abundant in stage T4. The expression appears to be weaker towards the maturation of somatic embryos. Preliminary results obtained by in situ RT-PCR indicate a putative expression of *PpRab1* in the outer layers of stage T2 of zygotic embryo development. In view of the proposed roles of Rab1 GTP-binding protein, the possible function of the protein encoded by *PpRab1* in embryogenesis is discussed.

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S7.6

THE DOUGLAS-FIR *LEAFY COTYLEDON1* (*PMLEC1*) GENE IS A TRANSCRIPTION FACTOR EXPRESSED DURING EARLY SOMATIC AND ZYGOTIC EMBRYOGENESIS AND IS INDUCIBLE BY STRESS AND HORMONES

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The objective of this study was to identify genes that function during conifer embryogenesis in order to increase our understanding of this process at the molecular level and for potential applications to somatic embryogenesis. In conifers, somatic embryogenesis protocols are not easily optimized because little is known about genes that function during zygotic embryogenesis. There are no molecular markers or gene expression profiles for comparison when embryogenic cell lines discontinue growth or stop developing. The ability to induce somatic embryogenesis from mature, vegetative tissues is still lacking. *LEAFY COTYLEDON1* is a CCAAT box-binding transcription factor that was first identified in *Arabidopsis thaliana*, and was shown to be critical for plant embryogenesis. LEC1 regulates embryonic processes by activating the transcription of genes required for development. It is responsible for specifying suspensor cell fate and cotyledon identity in early embryogenesis, and for acquisition of desiccation tolerance and expression of maturation-specific genes in late embryogenesis. Ectopic expression of *35S::LEC1* in *Arabidopsis* resulted in the spontaneous formation of somatic embryos from mature tissues. We describe the isolation and characterization of a *LEC1* homologue from Douglas-fir, *Pseudotsuga menziesii LEAFY COTYLEDON1*. The *PmLEC1* cDNA sequence was isolated using PCR and RACE-PCR. The putative Douglas-fir LEC1 protein shows 52% identity to AtLEC1. Northern blotting of Douglas-fir somatic and zygotic developmental stages shows that *PmLEC1* expression peaks during early embryogenesis. To identify factors that regulate *PmLEC1* expression, we treated mature seeds with stressors and hormones. It is expected that further work with *PmLEC1* will increase our understanding of the molecular biology of conifer embryogenesis and lead to improvements in somatic embryogenesis protocols.

S7.7p

ONTOGENY OF EARLY SOMATIC EMBRYO DEVELOPMENT IN THE CONIFER

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A fertilised egg is only partially enclosed by a cell wall, with its chalazal portion usually covered by the plasma membrane alone. A protoplast, therefore, comes close to resembling a zygote. We studied the cryptic potential of protoplasts of different lineages to form somatic embryos in the conifer, as represented by hybrid larch (*Larix x eurolepis* Henry). The lineages, obtained by discontinuous density gradient fractionation, consisted of (1) vacuolated non-cell regenerating suspensor protoplasts and cytoplasts, (2) mononucleate cell- and somatic embryo-regenerating protoplasts and (3) multinucleate non-cell regenerating protoplasts. The protoplasts were immobilised in thin films of alginate and cultured on embryo-sustaining medium. With regard to multinucleate protoplasts and in analogy to the partitioning of free nuclei during early prophase in zygotic embryogenesis, it was hypothesised that compartmenting of the nuclei by cell wall formation might result in the direct inception of an embryo or embryogenic tissue. However, we found that a protoplast containing two, three or even four nuclei might undergo mitosis and cytokinesis, but only as a result of one of its nuclei dividing and entering the cell cycle and only when cell wall resynthesis preceded nuclear division. The ability to enrich fractions of potentially embryogenic cells and to follow their morphogenic behaviour from the single-cell to the mature-embryo stage should help in understanding whether or not somatic embryogeny, though lacking the free nuclear divisions characteristic of prophase, mimics the development of its zygotic counterpart.

S7.8p

PHOTOSYNTHETIC CHARACTERISTICS OF WILD CHERRY PLANTLETS CULTURED IN VITRO AND EX VITRO

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This study examined those factors that account for the poor plant performance including photosynthetic parameters of wild cherry (*Prunus avium L.*) plantlets in vitro and when are transferred and grown under ex vitro conditions for 2, 4, and 10 weeks. The maximum net photosynthetic rate (Pm) of plantlets grown in vitro was low and increased 4-6 fold after transfer to ex vitro conditions. There was no relationship between the photosynthetic performance of in vitro grown plantlets and the total chlorophyll (a+b) and a:b ratio present in leaf tissue. The leaf dry mass accumulation and leaf area of in vitro plantlets were lower than ex vitro and were correlated with their photosynthetic capabilities. However, the specific leaf dry mass (SLM) was higher than ex vitro, which is not consistent with the variations in Pm. The amount and activity of the carbon-fixing enzyme, Ribulose-1-5-biophosphate carboxlase/oxygenase (Rubisco) were measured. Both a low Rubisco activity and a reduced electron transport capacity of in vitro plantlets underlie the low Pm, although it is more likely that either Rubisco activity or amount determines Pm under ambient conditions. Since leaf nitrogen concentration of in vitro plantlets was significantly higher than plantlets grown ex vitro we assume that Rubisco activity rather than the amount of this enzyme, limits Pm of in vitro plantlets. Measurements of the CO2 concentration in the jars containing plantlets gave concentrations of 300 and 200 *f*Ýmol mol-1 during the photoperiod at a growth irradiances of 50 and 200 *f*Ýmol photon m-2 s-1 respectively, which are lower than the ambient CO2 concentration. This may also restrict growth and photosynthetic performance under in vitro condition.

Key words: micropropagation, photosynthesis, Rubisco, wild cherry, acclimation

S7.9p

SOMATIC EMBRYOGENESIS IN *PINUS PATULA* – PROTOCOL DEVELOPMENTS AND PRELIMINARY FIELD RESULTS

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Vegetative propagation offers a means of maximising the deployment of genetically improved material. A major problem in the deployment of *Pinus patula* through conventional cutting propagation techniques is the early onset of ontogenetic maturation in the hedge plants. This results in a decline in the rooting of cuttings and poorer performance in-field, limiting clonal propagation as a deployment strategy. Somatic embryogenesis (SE) is a potential tool in the realisation of this strategic approach. The amenability of embryogenic tissue to cryopreservation offers a means of overcoming hedge maturation constraints. As hedges age and the performance of the resulting cuttings declines, SE-derived clones can be drawn from cryostorage for substitution of the aging hedges with more juvenile material. However, SE in this highly recalcitrant species has had limited success with low induction frequencies and poor genotypic responses to maturation. Douglas-fir Cotyledon Revised (DCR) medium proved to be twice as effective in encouraging initiation compared to MSG (modified MS) medium. Family responses to initiation have also been observed. Multiple inductions and the cumulative cryostorage of successfully induced genotypes have addressed low initiation rates. In an effort to improve embryo maturation responses, various maltose and ABA concentrations have been tested. A combination of 90 gl^{-1} maltose and 10 mgl^{-1} ABA yielded twice as many embryos compared to the control (60 gl^{-1} maltose and 10 mgl^{-1}), but the conversion (hardening-off) was disappointingly low. The use of a dual-phase medium during the singulation process has also led to improved embryo yields. The embryos reached Stage 4, two weeks earlier than the control tissue, and showed a 40 % increase in well-developed embryos in responsive genotypes. Cuttings obtained from SE-derived hedges are being tested in preliminary field trials to assess the feasibility of this propagation technique.

S7.10p

REGENERATION OF TRANSGENIC AMERICAN CHESTNUT PLANTS FOLLOWING CO-CULTIVATION OF EMBRYOGENIC TISSUES WITH *AGROBACTERIUM TUMEFACIENS*

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An efficient *Agrobacterium tumefaciens*-mediated transformation protocol for stable integration of foreign genes into American chestnut (*Castanea dentata*) has been established. Somatic embryogenic tissues from different genotypes were used as target material. *A. tumefaciens* strain AGL1 harboring the plasmid pCAMBIA 2301 [carrying the neomycin phosphotransferase II (*npt* II) selectable marker and B-glucuronidase (*uid*A) reporter gene] was used for the co-cultivation experiments. Several factors influencing transformation efficiency were investigated, including antibiotic concentration, plant genotype and co-cultivation conditions (pre-co-cultivation period, co-cultivation length and use of acetosyringone). For development of this protocol, GUS activity was routinely measured 4 days after co-cultivation (transient expression). Geneticin at 50 mg/l was found to be sufficient to inhibit the growth of somatic embryogenic cultures and was used for selecting putatively transgenic tissues. The frequency of transformation using *A. tumefaciens* varied with chestnut genotype. A four-day pre-co-cultivation period did not increase the number of transformants for all lines tested, but did increase the number of escapes. Acetosyringone added at 100 µM to the *Agrobacterium* suspension and to the co-cultivation medium increased transformation efficiency when embryogenic tissues were co-cultivated for 3 days. Mature somatic embryos of transformants from 2 different genotypes were germinated and more than 100 transgenic plants are presently being acclimatized. Antibiotic selection, as well as the transformation protocol per se, decreased embryo conversion for some genotypes. Stable integration of marker genes was confirmed by PCR analysis and expression of the *uid*A gene was demonstrated by GUS assay of tissues from transformed lines. As part of an effort to restore the species to the forest, the establishment of this broad-spectrum transformation system will be useful for future studies on transferring potential anti-fungal genes conferring resistance to chestnut blight.

S7.11p

ADVENTITIOUS SHOOT REGENERATION FROM LEAF PIECES AND ROOTING OF *PRUNUS SEROTINA* IN VITRO CULTURES

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Prunus serotina Ehrh. is an economically important species for timber and sawlog production in the central and eastern United States. The wood is used for cabinetry, fine furniture, and veneer. The objective of this study was to regenerate and root adventitious shoots of *P. serotina* in order to establish a regeneration system for use in genetic transformation. Nodal sections were cultured on Murashige and Skoog medium supplemented with 4.44 μM 6-benzylaminopurine (BA), 0.49 μM indole-3-butyric acid (IBA), and 0.29 μM gibberellic acid (GA_3). In vitro leaf explants of three genotypes were placed on woody plant medium (WPM) supplemented with 0, 2.27, 4.54, or 6.81 μM thidiazuron (TDZ) in combination with 0, 0.54, 1.07, or 5.37 μM 1-naphthaleneacetic acid (NAA); and on WPM supplemented with 0, 4.44, 8.88, or 13.32 μM BA in combination with 0, 0.54, 1.07, or 5.37 μM NAA. Cultures were maintained either in continuous darkness for 5 weeks, or in the dark for 3 weeks and then transferred to a 16-h photoperiod. TDZ and the genotype had a significant effect on the number of shoots regenerated. The maximum mean number of shoots regenerated per explant (1.18 ± 0.22; square root mean) was obtained with 6.81 μM TDZ plus 1.07 μM NAA. The highest rooting (70%) was obtained with 2.5 μM IBA when shoots were maintained for 4 days in the dark on rooting medium prior to transfer to a 16-h photoperiod. Eighty-six percent of the plantlets survived acclimatization to the greenhouse. This report of a complete protocol for adventitious shoot regeneration, rooting, and acclimatization of black cherry plantlets is a key step for future research on clonal propagation and genetic transformation.

S7.12p

SOMATIC EMBRYOGENESIS IN LOBLOLLY PINE: IMPROVING INITIATION WITH ORGANIC ACIDS

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A major barrier to the commercialization of somatic embryogenesis (SE) technology in loblolly pine (LP, *Pinus taeda* L.) is recalcitrance of some high-value crosses to initiate embryogenic tissue and continue early-stage somatic embryo growth. Developing initiation and multiplication media that resemble the seed environment may decrease this recalcitrance. Stage-specific analyses of 26 organic acids (OAs) were performed weekly throughout the sequence of seed development for female gametophyte (FG) and zygotic embryo tissues to determine physiological concentrations present that may be beneficial if added to SE media. Tissue from two open-pollinated families, grown in different locations and years, was analyzed in triplicate by gas chromatography / mass spectrometry (GC/MS). Significant changes in OAs occurred over time, with both seed collections showing similar trends in change of organic acid content. Although concentrations were not always similar, embryo and FG generally showed similar patterns of change in OA content over time. The major OAs contributing to osmotic potential were malic early in seed development and oxalic late in seed development. A simple bioassay was used to evaluate potential growth-promotion of the individual OAs added to initiation or multiplication media at physiological concentrations. Nine vitamins and 25 OAs were screened. Compounds showing statistically significant increases in early-stage embryo growth were then tested for initiation of LP. Two vitamins and five organic acids including vitamins B12 and E, *f*Ñ-ketoglutaric, pyruvic, succinic, oxalic, quinic, and ascorbic acids produced statistically significant increases in early-stage embryo growth. When tested for improved initiation, vitamin B12, vitamin E, *f*Ñ-ketoglutaric, pyruvic, and succinic acids increased initiation when alone and when combined. Analyses of OAs in the seed environment coupled with a bioassay to screen potential media supplements for protocol improvement resulted in statistically significant increases in the LP embryogenic tissue initiation protocol and saved much time and expense.

S7.13p

SOMATIC EMBRYOGENESIS IN NORDMANN'S FIR (*ABIES NORDMANNIANA*) AND ITS POTENTIAL APPLICATION FOR CLONAL MASS PROPAGATION AND DEVELOPMENT OF CLONAL VARIETIES

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In conifer species, somatic embryogenesis (SE) is an interesting model system for investigation of patterns and control of embryogenesis. Additionally, it is the basic process for the establishment of biotechnological procedures for clonal propagation and transformation. Both aspects, the fundamental and the practical, will be presented using the example of *Abies nordmanniana*. *Abies nordmanniana* has an enormous commercial importance for Christmas tree production in Germany and Europe. It is exclusively grown from seeds harvested from natural populations in the Caucasian mountains. Clonal varieties would help to improve the quality of trees and the cultivation characteristics considerably. SE-based propagation methods are expected to be a realistic possibility to solve these problems within the next decade. In *Abies* spp., SE has been reported for the first time 1989. In the recent years the developmental patterns were described in detail and the protocols for initiation, proliferation, maturation and germination were improved. The characteristic developmental patterns, the control of embryo development as well as the advantages and limitations for commercial applications will be discussed.

S7.14p

FIRST STEPS OF PROGRAMMED CELL DEATH MONITORED BY THE ACTIVITY OF THE MITOCHONDRIAL K+ATP CHANNEL IN THE EMBRYOGENIC CULTURES OF DIFFERENT CONIFEROUS SPECIES

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During the proliferation of embryogenic cell masses the first way of programmed cell death was described in [2]. The aim of the present study was to monitor the energy status of proliferating embryogenic cell masses and to characterise the K^+_{ATP} channel in mitochondria isolated from embryogenic cultures of three different coniferous species (*Picea abies, Abies cephalonica* and *Pinus nigra*). For fulfilling these aims, we measured the amount of glucose-6-phosphate and ATP at regular time intervals during proliferation, in order to select the time in which the energetic level of cells is high (6^{th} day after transfer) and, hence, to isolate mitochondria. Crude mitochondria, from embryogenic cultures, showed typical features of intact and functional plant mitochondria, such as integrity (ca. 90%), basal oxygen uptake (ca. 100 nmol/min per mg of protein), respiratory control ratio (ca. 2.5) and partial KCN-resistant respiration. The characterisation of the mitochondrial K^+_{ATP} channel was performed by measuring in isolated *P. abies* mitochondria: the K^+-inward activity (KCl-induced electrical potential dissipation in energized mitochondria, or swelling in de-energized mitochondria resuspended in a KCl-based medium); the spontaneous and cyclosporin A-stimulated K^+-outward activity (electrical potential building-up in de-energized mitochondria). The characteristics of the K^+_{ATP} channel from embryogenic cell masses were very similar to those previously described for a channel of soybean mitochondria [1]. In particular, K^+-inward fluxes appeared to be enhanced; both K^+-inward and outward activities were inhibited by ATP, stimulated by diazoxide, and modulated by reducing agents (DTE). These results strongly suggest that the K^+_{ATP} channel is also involved in the manifestation of PCD during the proliferation of embryogenic cell lines of coniferous species.

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S7.15p

RECOVERY OF SOMATIC EMBRYOS AND PLANTS FROM PROTOPLAST CULTURES OF SAWARA CYPRESS (*CHAMAECYPARIS PISIFERA*)

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Sawara cypress (*Chamaecyparis pisifera*) is native to Japan. It is a very important conifer in horticulture and the wood is also used for furniture manufacture. Previously we have shown that plant regeneration of 2 species of the genus *Chamaecyparis* (*C. obtusa* and *C. pisifera*) from embryogenic cultures derived from immature embryos (Maruyama et al. 2005a, 2005b). This report demonstrates that somatic embryos and plants can be recovered from protoplasts derived from embryogenic suspension cultures of *C. pisifera*. The donor suspension culture for protoplast isolation was established from an immature embryo and maintained using a liquid medium containing 2,4-D and BAP. Protoplasts were isolated from the suspension cultures using an enzyme mixture containing Cellulase RS, Driselase along with 0.6 M mannitol. The viability, as determined in FDA stainings, was more than 90%. Freshly isolated protoplasts were cultured in liquid medium containing 0.6M mannitol using 96 well plates. After 1 month of culture, protoplast-derived colonies were proliferated and transferred onto solid medium containing 10mM glutamine, 6% maltose, 10% PEG (MW: 7,300 - 9,300), 50 μM ABA and 0.2% activated charcoal. After 30 to 40 days of culture on the medium, the first somatic embryos were detected. Mature cotyledonary embryos were transferred onto germination medium. Some of these developed into plants that were transferred to soil. This is the first report on plant regeneration from *C. pisifera* protoplasts.

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S7.16p

EVALUATION OF GENETIC STABILITY IN CRYOPRESEVED EMBRYOGENIC CULTURES OF *PINUS PINASTER*

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Somatic embryogenesis and cryopreservation have become significant components of reforestation and breeding strategies in conifers. Maritime pine (*Pinus pinaster* Ait.) is the main forest species in Portugal and an *in vitro* system consisting of somatic embryogenesis coupled to cryopreservation has already been established for this species. Dimethyl sulfoxide (DMSO), used in cryopreservation, has been described as a mutagenic compound. It is desirable to assess the genetic integrity of embryogenic cultures/plants surviving cryogenic storage to determine if they are "true to type" after cryopreservation. SSRs have been described as an ideal system for assessing the mutability in somatic embryogenic tissues. The main objective of this work is to establish an efficient marker system using SSRs, to evaluate the stability of cryopreserved embryogenic cultures and subsequent regenerated emblings of maritime pine. Nuclear microsatellite DNA markers of simple sequence repeat (SSR) loci, isolated from *P.pinaster*, were used to test for genetic stability in cryopreserved and non-cryopreserved embryogenic tissue samples. The fragment analysis was done by Capillary sequencer Beckman Coulter (CEQ^{TM}8000) and the fragment length of the PCR-products were evaluated by CEQ^{TM}8000 Genetic Analysis System Software. Up to now, conditions for DNA extraction, PCR and fragment analysis have been optimized for 5 SSRs loci (FRPP91, FRPP94, ITPH4516, PtTX3107, PtTX3116). The application of established conditions has been initiated and seven different embryogenic lines are under evaluation for genetic stability. The obtained results will be presented.

S7.17p

TRANSFORMATION AND MATURATION STUDIES WITH EMBRYOGENIC TISSUE OF *PINUS RADIATA*

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The genetic transformation of pine, while successfully achieved with a variety of species, remains very inefficient. For embryogenic cell lines of radiata pine competency for transformation through either Biolistic or Agrobacterium-mediated methods, and competency for maturation, are not always positively correlated. To investigate this relationship, over 20 genotypes were used in Biolistic® transformation experiments, followed by attempts for embryo maturation, either prior to or following cryopreservation. Transformation efficiency varied from 0 to 35% in lines not previously cryopreserved, and from 0 to 2% in those recovered from cryopreserved embryogenic tissue. Maturation was achieved in the majority of the cell lines tested. However, for the highly transformable cell line (73:03) no maturation was observed. Conversely, for several cell lines good maturation was achieved but they were not transformable. The efficiency of two promoters in promoting the production of transgenic lines was also evaluated using the npt II selection gene under the control of either the maize ubiquitin promoter or the 35S CaMV promoter. Results from 400 bombarded Petri dishes showed that the ubiquitin promoter enhanced transformation efficiency. The implications of these results for forest biotechnology will be discussed.

S7.18p

SOMATIC EMBRYOGENESIS OF *LIRIODENDRON* HYBRIDS (*L. CHINENSE* (HEMSL.) SARG.¡Á *L. TULIPIFERA*) AND LARGE SCALE EMBDLING PRODUCTION

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It was of great using value and practical significance for mass propagation *Liriodendron* Hybrids (*Liriodendron chinense* (HEMSL.) SARG. ¡Á *L. tulipifera*), an excellent and important tree species both for landscape and industrial production in southern China. The supplement of the hybrids was short of demand, for the present situation that the parental resource was rare and the hybrids seed production efficient was low by conventional hybridization. We demonstrated an example for hybrids mass propagation by somatic embryogenesis and large scale plant regeneration based on a 40 year¡¯s conventional hybridization breeding program, supplying 300 millions regenerated plants (with more than 10 lines) through somatic embryogenesis annually. We created an interspecies hybrid in 1963, which shown obvious heterosis both on growth and adaptation, by select the parents material from *Liriodendron chinense* (HEMSL.) SARG. and *L. tulipifera*. Somatic embryogenesis was successfully done with the immature zygotic embryos as explants during 1999-2002, and extended to large-scale production during 2003-2005. The genes which control the dedifferentiation and redifferentiation were expressed and the cell totipontency had been fully realized when the explants were regulated by different plant hormone, basal medium and cultural condition. It was found that the developmental stage of the explant played a key role in the experiment. The aggregate samaras were picked weekly and it was found that the immature zygotic embryos from global embryo to cotyledon were easily implemented somatic embryogenesis of *Liriodendron* hybrids. The culture condition, especially the light condition, was an important factor for the embryogenesis. The embryogenic callus could be only induced under the dark condition, and once the embryogenic callus was formed it should be cultured under the light condition to control its growth. The culture condition would also play a significant role on the multiplication of the somatic embryos. The light condition was very important for the normal development of the somatic embryos, while under the dark condition the embryos couldn¡¯t multiply any more and even developed abnormally into yellow embdlings, abnormal embdlings. In order to meet the need of the nitrogen source at different developmental stages, the basic medium for each developmental stage had been screened by regulating the major salts concentration of the MS medium. We used MS medium at all developmental stages nearly in case of the induction stage, in which the 1/2 MS medium was used, to meet the demand of the nutrient. On the other hand, the inorganic salt could also change the osmotic potential indirectly. Embryogenesis was induced after the explants dedifferentiated into embryogenic callus with 2 mg¡¤L-1 2,4-D; But the concentration of 2,4-D should be reduced or removed at all at the second developmental stage in time. NAA (6mg¡¤L-1)could also help to induce the callus but not embryogenic callus. ZT could counteract the aftereffect resulted from the high concentration of auxin and induce the embryogenesis if added properly. As soon as the callus becomes much dense, it should be transplanted in the medium with ZT. Some abnormity of the embryo development could be restrained effectively under the action of ABA at physiological level. GA could promote the elongation growth of the regenerated plants. Proper amount of activated charcoal in the medium not only played an important role for the growth of the young embdlings, but also promoted the formation of the root. Histological study on the origin and development of somatic embryos, it was found that somatic embryos were mostly developed from single embryogenic cell that possessed the ability of embryoid occurrence from the surface of the

callus. At the early development stages, the proembryos were limited in a boundary of thick cell wall. Plantlet regenerations were developed from global embryo, heart-shaped embryo, torpedo-shaped embryo and cotyledon embryo, in which the V-shaped vessel vascular bundles and shoot tip meristem and root tip meristem were observed. The thick cell wall formed when single embryogenic cell developed. The somatic embryo formed mostly from single cell superficially, thus when the relation between the cell and the environment was cut down, the cell polarity would be constructed more easily. At this time, the suspensor perhaps was the only way for the nutrient absorption and exchange. The significant character of the somatic embryo was its polarity, that is to say, the radical and the plumose emerged at the early stage of the somatic embryogenesis. The first mitosis of the embryogenic cell was an unequal mitosis mostly, then the top-cell (then divided into multi-cell pre-embryo) and base-cell (then divided into suspensor) formed in the end with the distinct polarity. Starch played a key part in the development of the somatic embryogenesis too, such as those accumulated in the cotyledon may be helpful to the form of embryogenic callus. Now we can find that the content of the starch would increase or reduce during the somatic embryos development. There were two types callus: embryogenic callus and non-embryogenic callus. The major features of each one was observed by means of scanning electron microscopy technique. We found that embryogenic cell was often globular and equal while the non-embryogenic callus was unequal significantly. The embryogenic tissue cultured from *Liriodendron* Hybrids had a significant polarity and relative independence. The somatic embryo, especially the mature somatic embryo, would become incompact with embryogenic callus and fall off easily. It was found that the embryogenic cell was regular and abundant in cell organelle while the nonembryogenic cell and torpedo cell contained many paramural bodies by studying the different developmental stage of the embryogenic cell, nonembryogenic cell and somatic embryo through transmission scanning electron microscope, which indicate that there was a comprehensive signal transconduction and material transportation at this two stages. The cotyledon somatic embryo and the zygotic embryo have much similarity from the ultrastructure, in which there were full of lipid body and protein body and degenerated cell organelle. The genetic stability of the regenerated plantlets has been studied by chromosome numeration and it was found that these somaplants were diploid mostly, while only one of the somaclones to regenerate plants with tetraploid karyotype. We use this system to supply 300 millions regenerated plants (with more than 10 lines) through semi-solid medium culture annually. Furthermore, we tried more efficient system by means of bioreactor system.

S7.19p

MAJOR FACTORS CONTRIBUTING TO MICROGRAFTING SUCCESS RATE OF MATURE MARITIME PINE

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Micrografting of bud meristems onto juvenile rootstocks is currently the best option for efficient *in vitro* establishment of mature trees in maritime pine because most genotypes are responsive. The interest of this technique for both practical application and basic research mainly arises from its potential to rejuvenate and/or reinvigorate and thus to restore the morphogenetic competence of the mature plant material. Unfortunately, micrografting is a technically complex procedure with quite low throughput. Full practical benefits are thus expected only when optimal conditions for micrografting maritime pine are defined. The objective of this work was to identify major factors contributing to the final Micrografting Success Rate (MSR, scion development observed 2 months after micrografting) of 1-year-old grafts from mature (15 years old), field-tested donor trees (6 genotypes). Season when buds are collected was apparently the main factor affecting MSR in strong interaction with clone and operator. Results were greatly improved in late winter (89%) compared to early spring (64%), summer (49%) or autumn (48%). Interestingly, minimal differences were observed during the best sampling season among genotypes ($\leq$ 13%) or operators ($\leq$ 10%). Additional variations factors with more limited impact on MSR were revealed such as rootstock chronological age (+12% MSR using 3-month-old vs. 2 month-old seedlings) and bud position on grafted mother plant (+9% using buds collected on secondary axes vs. principal axis). No significant difference was observed between terminal (54%) and axillary buds (57%). Bud storage in chilling condition (4°C) for 1 month prior to micrografting did not improve MSR except in autumn (+12%). The newly defined conditions will improve micrografting efficiency of maritime pine and thus facilitate *in vitro* establishment of adult trees.

S7.20p

ZYGOTIC AND SOMATIC EMBRYO MORPHOGENESIS IN *PINUS PINASTER*: A HISTOLOGICAL AND HISTOCHEMICAL APPROACH

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A comparative study on morphogenesis and accumulation of storage protein and starch in somatic versus zygotic embryos during maturation in maritime pine (*Pinus pinaster* Ait.) was performed. Maturation media included 120 µM ABA and 9 g/L gelrite, testing glucose, sucrose or maltose at 44, 88, 175 or 263 mM in the presence or absence of 6% (w/v) PEG 4000 MW. Treatments involving 44 or 88 mM carbohydrate produced 0-1 cotyledonary somatic embryo. PEG induced a low number of cotyledonary somatic embryos, which showed incomplete development, large intercellular spaces and vacuoles. Embryo response to 175 or 263 mM maltose treatments was negative in the presence of PEG but positive in its absence. The higher maltose concentration, however, induced large intercellular spaces. Sucrose at the highest concentration induced lower number and less developed cotyledonary somatic embryos as compared to 175 mM. These results suggest that the effect of carbohydrate source in this species is partially osmotic. Morphogenesis was similar between zygotic and somatic embryos. Starch granules followed similar accumulation pattern in zygotic and somatic embryos. In zygotic embryos, protein bodies appeared later and were smaller and fewer than in somatic embryos, as in PEG-treated somatic embryos. The lowest dry weight was detected in zygotic embryos (0.33 mg/ embryo) followed by 175 mM maltose treated embryos (0.61 mg/embryo). The lower starch content was found in cotyledonary zygotic (1.7 µg/embryo) and somatic embryos produced on 175 mM maltose (4.3 µg/embryo) or 263 mM glucose (5.7 µg/embryo), which may be good alternative treatments for maturation of this species. Storage protein levels can be potential indicators for the selection of good quality somatic embryos. Embryo protein content is being analysed by two-dimensional electrophoresis in order to confirm this hypothesis.

S7.21p

SOMATIC EMBRYOGENESIS AND CRYOPRESERVATION OF *SAPINDUS MUKOROSSI* GAERTN

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Sapindus mukorossi, deciduous high tree have grown naturally in Southern Korea, China, and Japan Island for medicinal, street and gardening trees. In recently this trees have been disappeared rapidly because the developing Southern region in Korea. It is important to conserve the genetic resources of *S. mukorossi*. There are three methodologies for genetic resources; in situ, ex situ, and in facilities. In facilities cryopreservation methodology is useful method by somatic embryos. The somatic embryogenic callus of *S. mukorossi* is induced in B5 medium without phytohormone transferred by the B5 medium with the growth hormone of 0.1 mg/l BA and 0.01 mg/l 2,4-D during one month culture. Suspension culture is carried for mass propagation of somatic embryos in a bioreactor. The cell growth showed that B5 liquid medium with 0.1 mg/l BA and 0.01 mg/l 2,4-D shows the best growth. Two cryopreservation methods, encapsulation and vitrification are used. The vitrification methods have better survival rate than encapsulation. The vitrification with PVS2 solution at 0oC for 10~20 minutes has more than 80% survival rate. After stocked the vitrificated somatic embryos in liquid nitrogen more than one day, the cryoprserved embryos are not genetic variation by RAPD.

S7.22p

PLANTLET REGENERATION OF *CAMPSIS GRANDIFLORA* (THUNB.) SCHUMANN BY AXILLARY BUD CULTURE

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To establish a reliable micropropagation method axillary buds were collected from young plants and were surface-sterilized and dissected into 2-3cm lengths. The explants were cultured on Woody Plant Medium containing a combination of BAP(0, 0.2, 0.5, 1.0, 2.0 mg/l) and NAA(0, 0.01, 0.1 mg/l). After 6 weeks culture 60% of axillary buds grew into new shoots at the highest rate on media containing BAP 0.2mg/l and NAA 0.1mg/l. For multiple shoots production the concentrations of BAP (0.5 and 1.0 mg/l) contained in WPM were compared by culturing new shoots from axillary buds. Average number of multiple shoots were 5 on higher concentration (1.0mg/l) and 3 on lower concentration (0.5mg/l). Some cultures showed rooting.

S7.23p

AGROBACTERIUM-MEDIATED RMCE APPROACH FOR GENE REPLACEMENT

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We developed a site-directed integration (SDI) system for *Agrobacterium*-mediated transformation to precisely integrate a single copy of a desired gene into a predefined target locus by recombinase-mediated cassette exchange (RMCE). We produced site-specific transgenic plants from 4 target lines and examined expression of the transgene in T1 site-specific transgenic plants, which were obtained by backcrossing. We found that site-specific transgenic plants from the same target lines showed approximately the same level of expression of the transgene. Moreover, we demonstrated that site-specific transgenic plants showed much less variability of transgene expression than random-integration transgenic plants. Interestingly, transgenes in the same direction at the same target locus showed the same level of activity, but transgenes in different directions showed different levels of activity. The expression levels of transgene did not correlated with those of the target gene. Our results showed that the SDI system could benefit the precise comparisons between different gene constructs, the characterization of different chromosomal regions and the cost-effective screening of reliable transgenic plants.

S7.24p

COULD BE APPLIED AN IN VITRO CLONING PROCESS FOR BREEDING AND MASS PROPAGATION? ADVANCES ACHIEVED IN GENUS *PINUS*

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Several approaches have been developed and adjusted in an *in vitro* cloning process that has the competence to be applied to species of Genus *Pinus*, however several modifications must be done in order to accomplish full process. Induction as well initiation of pine somatic embryos at different percentages were obtained in immature zygotic embryos in species belonging to subgenera *Diploxilon* and *Haploxilon*, by means of different PGR including abscisic acid (ABA); establishment of proliferation can be reached in 100% of the genotypes by means of culturing onto different media (suspension cultures is also useful for at least one year), this methodology includes an easy action that is interchanging embryogenic masses among the media, these strategies allow to fulfil all genotype requirements in both subgenera. At this step, a different approach other then cryopreservation can be applied to immature somatic embryos in order to maintain the embryogenic capacity, as simple maintenance of suspension cultures at 4° C. Both, suspension cultures and preservation at 4° C were applied successfully to phylogenetic distant species (*Pinus maximartinezii* and *P. sylvestris*); regarding to Scots pine, this method can be useful for genetic transformation by biolistic protocols since can be regenerated 50 mature somatic embryos per gram fresh weight (PGFW). However, maturation process seams to be different depending on the subgenera, for instance, in a species belongs to subgenus *Diploxilon* [Scots pine (*P. sylvestris*)] a low ammonium to nitrate relation (10/90) plus maltose and activated charcoal are the targets to induce the development of somatic embryo, and later adding ABA to the same medium compound (lacking of activated charcoal), full maturation is achieved; in which are included genotypes that are considered producers of aberrant type embryos; this result demonstrates those genotypes have only physiological difficulties instead of genetic concerns. The appliance of the methodology seams to be inadequate on (*P. maximartinezii*, specie belongs to subgenus *Haploxylon*, where somatic embryos are stimulated to proliferate, in case of zygotic embryo induction and initiation of embryogenic tissue is achieved. Concerning to the following steps, normal germination of several thousand of embryos, well rooting and development of plants were achieved in Scots pine in most of the genotypes tested (up than 80%) belonging to more than 95% of the mother trees tested, where that percentage were also reached in adaptation of emblings, where the plants shown long roots, and development of inter nodes after being transferred to growth chambers. The protocol developed for Scots pine can be useful for genetic breeding by the basis described above, and mass propagation is not a challenge any more since can be produced from 5 to 1500 mature somatic embryos PGFW.

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S7.25p

PLANTLET REGENERATION OF *SCHISANDRA CHINENSIS* (TURCZ) BAILL. BY SOMATIC EMBRYOGENESIS

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Plants were regenerated via somatic embryogenesis from zygotic embryo explants isolated from mature seeds. Merkle and Sommer¡¯s medium with 2,4-D(9.04M) and zeatin(0.09M), was effective for induction of embryogenic callus. The embryogenic callus induced on Merkle and Sommer¡¯s medium with 2,4-D(9.04M) and zeatin(0.09M) showed development of the maximum number of somatic embryos when transferred to MS medium free of PGRs. Emblings were successfully hardened in the soil.

S7.26p

ANALYSIS OF GENE EXPRESSION DURING EARLY SOMATIC EMBRYOGENESIS IN *PINUS RADIATA*: MOLECULAR CHARACTERIZATION OF A TRITHORAX GENE

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Somatic embryogenesis (SE) is an efficient technique for plants propagation. We have implemented this method of propagation in *Pinus radiata*. Little knowledge exists about molecular events and genes that participate during the growth and development of somatic embryos. For this reason cDNA-AFLP was used to identify genes expressed during SE in *Pinus radiata*. The early SE was initially studied, because these stages are crucial for successful completion of the overall process. Three stages of somatic embryo development were analyzed: Proembryo, late proembryo and early embryo. No-embryogenic tissue was used as control. In our study, almost 4000 transcript-derived fragments (TDF) were amplified and 60 TDF that are expressed in specific stages or with differential patron during the stages analyzed were sequenced. 50% of TDFs did not show any homology to sequences with known functions, the rest showed homology to genes involved in metabolism, programmed cell death, stress response and transcription activation. We confirmed the expression of 5 TDFs via RT-PCR. A TDF with homology to a Trithorax gene was found and then the full-length cDNAs was isolated. The product of the Trithorax gene has been identified genetically as a positive regulator of homeotic genes in other species. The identification of genes activated during SE can be used as a tool to improving this process in *Pinus radiata*.

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S7.27p

INSIGHTS INTO MECHANISMS FOR ENHANCED GROWTH OF TRANSGENIC POPLAR EXPRESSING PINE GLUTAMINE SYNTHETASE: INCREASED GLUTAMINE CONTENT OF LEAVES SIGNIFICANTLY ENHANCES TRANSCRIPTION OF ANTHRANILATE SYNTHASE (AS) ALPHA SUBUNIT

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When compared with non-transformed controls, hybrid poplar (*Populus tremula* L. X *P. alba* L., clone--INRA 7171-B4) expressing pine cytosolic glutamine synthetase (GS1a) show significant improvement in plant growth, enhanced GS activity, increased levels of free glutamine, and enhanced nitrogen utilization efficiency (Gallardo et al, 1999; Fu et al., 2001; Man et al., 2005). Improved growth of GS transgenics prompts us to examine potential roles of glutamine in global nitrogen metabolism and in the mechanisms regulating plant growth. The initial reaction in the pathway leading to production of indole-3-acetic acid (IAA) in plants is the reaction of chorismate and glutamine to produce anthranilate, catalyzed by anthranilate synthase (AS). Using RT-PCR, we have shown that transcription of the alpha subunit of AS is enhanced in leaves of GS1a transgenic poplar. Furthermore, exogenous feeding of detached tobacco leaves with 30 mM glutamine significantly enhanced transcription of the AS alpha subunit. In preliminary studies, enhanced IAA levels in GS1a transgenic poplar and in detached tobacco leaves supplied with exogenous glutamine were observed. The possible role of glutamate/glutamine ratio in moderating nitrogen/carbon metabolism and the possible function of glutamine in promoting plant growth will be presented.

S8. Biotechnology and metabolic engineering of wood formation in trees

S8.1

USE OF AN *ARABIDOPSIS* CAD MUTANT TO DETERMINE THE FUNCTION OF CADS OF *ARABIDOPSIS* AND TREE ORIGIN

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In Angiosperms, the lignin biopolymer is composed mainly of guaiacyl (G) and syringyl (S) units. These units are derived from coniferyl and syringyl alcohols, also named monolignols. Cinnamyl alcohol dehydrogenase (CAD), the last enzyme of the monolignol-specific pathway, is responsible for the reduction of hydroxycinnamaldehydes to hyroxycinnamyl alcohols. CAD genes belong to multigene families in plants; For example, nine genes are present in the *Arabidopsis* genome (Goujon et al, 2003). The expression profile of two of them, AtCAD-C and AtCAD-D, is compatible with a major role in stem lignification (Sibout et al, 2003) but only a double mutant (*Atcad cd*) affected in these two CAD shows reduced lignin content and a modification of lignin composition with significant levels of cinnamaldehydes incorporated instead of cinnamyl alcohols (Sibout et al, 2005). In order to determine if other CAD proteins possess the ability to reduce cinnamaldehydes, this double mutant has been used for complementation studies. Molecular complementation of this *Atcad cd* mutant with pine CAD, poplar CAD and SAD as well as with the 9 *Arabidopsis* CAD genes under the control of the CAD-D promoter suggests different abilities of these genes/proteins in the production of syringyl-lignin.

Goujon et al (2003) Plant Physiol. Biochem. 41 : 677-687
Sibout et al (2003) Plant Physiol. 132 : 848-860
Sibout et al (2005) Plant Cell : in press

S8.2

CHARACTERIZATION OF A TEMPERATURE-SENSITIVE MUTANT OF *ARABIDOPSIS*, *lig*, WHICH EXHIBITS ABERRANT LIGNIN DEPOSITION AND GROWTH DEFECTS

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A temperature-sensitive mutant of *Arabidopsis thaliana*, *lignescens* (*lig*), was isolated by screening with adventitious root formation as an index phenotype and characterized by aberrant accumulation of lignin at higher temperatures (Duan and Sugiyama 2001, J Plant Res 114: s109). In this study, we characterized the *lig* mutant for elucidation of the regulatory mechanism of lignin synthesis. Whereas the *lig* seedlings grew normally at 18°C (permissive temperature), their root growth was inhibited severely at 28°C (restrictive temperature). When the *lig* seedlings were grown at 18°C and then transferred to 28°C, the lignin content started to increase 2 days after the temperature shift. Phloroglucinol-HCl staining and Mäule staining showed accumulation of guaiacyl lignin in the sub-apical region of the root of the *lig* seedling. A map-based approach identified a missense mutation in a putative serine/threonine kinase gene of the *lig* genome, which might be responsible for the *lig* phenotype. To gain insight into the molecular mechanism of aberrant lignin accumulation in the *lig* mutant, we investigated effects of the *lig* mutation on expression patterns of several genes directly involved in or indirectly related to lignin formation, including *AtPAL1* encoding phenylalanine ammonia lyase, *AtCCR1* and *AtCCR2* encoding cinnamoyl-CoA reductase, *AtCAD-D* encoding cinnamyl alcohol dehydrogenase, and *CESA3* encoding cellulose synthase. Exposure of the *lig* seedlings to 28°C decreased the expression of *CESA3*, and increased the expression of *AtPAL1*, *AtCCR2*, and *AtCAD-D*. Based on these results, a possible function of the *LIG* gene in the regulation of lignin synthesis will be discussed.

S8.3

STRATEGIES FOR IDENTIFYING DETERMINANTS OF SECONDARY CELL WALL FORMATION USING MODEL SYSTEMS

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The physiochemical properties of wood are determined by the composition of secondary cell wall polymers and the molecular interactions that exist amongst them. To learn more about the molecular mechanisms involved in secondary cell wall synthesis, we have first used a functional genomics in the model xylogenesis system in *Zinnia elegans* to obtain hundreds of new gene sequences corresponding to the stages of secondary cell wall formation in tracheary elements and followed their temporal expression patterns. A bioinformatic search led to the identification of *Arabidopsis* and poplar orthologs for each *Zinnia* gene. Approximately 60 homozygous lines of *Arabidopsis* mutants were identified and subsequently subjected to mid-throughput phenotypic analysis including *in vitro* growth, growth under different light regimes, microscopic analysis of vasculature, susceptibility to wall degrading enzymes, and resistance to *Ralstonia solanacearum*, a bacterial vascular pathogen. Using this strategy we have identified several mutants with varying wall-related phenotypes including poorly lignified stem fibres, ectopic lignification in stems, reduced floral stem elongation, altered susceptibility to wall degrading enzymes, and increase tolerance to pathogens. The detailed characterisation of some of the most interesting mutants, now underway in our laboratory, will be discussed.

Pesquet et al. Plant. J. (2004)

S8.4

USE OF POPLAR AND *ARABIDOPSIS* MODEL SYSTEMS TO IDENTIFY GENES INVOLVED IN WOOD FORMATION

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The plant cell wall is a vital structural component regulating cell volume and shape, providing mechanical support, mediating cell-cell interactions, and provides the raw materials for a number of important processes. However, relatively little is currently understood about the genetic regulation of cell wall and wood formation in plants. We have adopted an approach utilizing transcript profiling of the Poplar cambium to identify potential novel regulators of wood formation. The Poplar cambium has distinct ontology of cellular development making it an ideal model system to study developmental processes. Despite the advantages, it is time consuming to analyse large numbers of genes in this species, so closely related orthologs are first studied in *Arabidopsis*. To date work has focused on characterization of the Poplar orthologs of the *Arabidopsis EPC1* gene. *Arabidopsis EPC1* knockouts result in plants with a reduced cell-cell adhesion and this results in an increase in secondary growth in hypocotyl tissues. This is significant as it potentially provides a tool to modulate wood biomass production in trees. The Poplar *EPC1* orthologs have been cloned and approaches are currently in progress to determine their function during wood formation. Further target genes which function during xylem formation and secondary growth in *Arabidopsis* are being studied using a variety of techniques including morphological analysis, reporter based studies and FT-IR to characterize chemical alterations due to single gene knockouts. This approach integrating *Arabidopsis* and Poplar model systems provides a rapid method to determine gene function.

S8.5

NEW MYB TRANSCRIPTION FACTORS FROM *EUCALYPTUS* XYLEM REGULATE SECONDARY CELL WALL FORMATION AND LIGNIN BIOSYNTHESIS

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Wood which is mainly composed of secondary cell walls, represent a major part of plant biomass on earth and the fifth most important product of world trade whose demand is continuously increasing. Lignin is a major component of these secondary cell walls allowing mechanical support and efficient conduction of water and solutes over long distance within the vascular system. For the pulp and paper industry, lignin is an undesirable polymer, which has a negative impact on both pulp quality and yield and has to be removed from cellulose by costly and polluting chemical treatments. On the other hand, an increase of the lignin content could be beneficial to give harder wood-derived material or provide more energy during wood combustion. Despite the outstanding importance of lignin at the biological, environmental and economical levels, the molecular mechanisms governing its formation are far from being understood. As part of a programme aimed at identifying major regulators of lignin biosynthesis, we have cloned and characterised two transcription factors of the MYB family (*EgMYB1* and *EgMYB2*) from a cDNA library prepared from *Eucalyptus* differentiating xylem. Both transcription factor are highly and preferentially expressed in *Eucalyptus* xylem and are able to bind specifically to the promoters regions of lignin biosynthetic genes. *EgMYB2* to activate their transcription whereas *EgMYB1* acts as a repressor. Transgenic tobacco plants overexpressing *EgMYB2* exhibited a dramatic increase in xylem secondary cell wall thickness as well as alteration of the lignin profiles. The changes in the lignin monomeric composition are a consequence of differential activation of individual genes of the lignin biosynthetic pathway. *EgMYB2* is therefore a positive regulator of lignin biosynthesis and secondary cell wall formation which render it a good tool for breeding or biotechnological programmes aimed at improving wood properties.

S8.6

METABOLIC PROFILING OF THE CINNAMATE/MONOLIGNOL PATHWAY BY THE USE OF STABLE-ISOTOPE-DILUTION METHOD

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The cinnamate/monolignol pathway which supplies precursors for various phenylpropanoid compounds such as lignins, lignans, norlignans, and flavonoids plays central roles both in cell wall formation and heartwood formation in trees. During the last decade, substantial progress has been made in studies of the pathway, mostly due to development of molecular biological techniques. For example, a novel pathway towards syringyl lignin via 5-hydroxyconiferylaldehyde was elucidated, and a transgenic aspen with less lignin and more cellulose contents was reported. However, comprehensive mechanisms for the control of the pathway remain to be elucidated. In the post-genomic era, to understand the comprehensive mechanisms, comprehensive analyses such as transcriptomics, proteomics and metabolomics are becoming key strategies. However, accurate and comprehensive quantitation methods for whole metabolites, i.e. true metabolomics, have not yet been established. Hence, as a first step, we focused on the cinnamate/monolignol and lignan pathways and established a comprehensive quantitation system for the metabolic intermediates of the pathways. For this purpose, we employed a stable-isotope-dilution method, because this is the most reliable quantitation method. First, we synthesized deuterium-labeled and unlabeled standards for each of more than 30 metabolites in the pathways, and established standard calibration curves for the target compounds. Next, the system was successfully applied to characterization of the pathways in *Carthamus tinctorius* (safflower) seeds where biosynthesis of both lignins and lignans increases rapidly during maturation. In addition, ^{13}C-labeled precursors were administered to the seeds, and ^{13}C incorporation into the downstream metabolites were measured comprehensively. These metabolic profiling data during the seed maturation were analyzed together with time-dependent gene expression data obtained by real-time PCR, resulting in identification of several cDNA clones involved in lignin and lignan biosynthesis. In addition, the presence of a biosynthetic route specific to lignan biosynthesis among many possible routes in the cinnamate/monolignol pathway was strongly suggested.

S8.7

CARBOHYDRATE ACTIVE ENZYMES IN THE HYBRID ASPEN XYLEM

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Wood fibers constitute a renewable raw material, which can be processed using enzymes during post-harvest processing or – in the future – by *in vivo* fiber engineering. A number of different enzyme systems contribute to the cell wall formation and the resulting fiber structure and properties in trees. While lignin biosynthesis has become relatively well understood, this is not the case for the synthesis of key structural carbohydrates of the fibre walls. We have used a functional genomics approach to identify carbohydrate-active enzymes involved in xylem development in hybrid aspen, *Populus tremula x tremuloides* (1-2). Bioinformatic analyses using sensitive tools developed for carbohydrate-active enzymes allowed the identification of a large number of enzymes in eight families of glycosyl transferases and glycosyl hydrolases (or transglycosylases)(3). Among these were a set of four xylem specific *CesA* genes, one of which was even more highly expressed in tension wood (4). Further analysis of the genome sequence of *Populus trichocarpa* led to the identification of 18 different genes encoding *CesA* isoforms (5). This was a surprise as the *Arabidopsis* genome apparently only contains 10 *CesA* genes. The gene that, according to the microarray analysis, was most highly expressed in the early secondary cell wall tissues of hybrid aspen, encodes a protein with no known function. A fragment of the protein has been expressed in *E. coli*, followed by the production of specific antibodies in rabbits and chicken. Data obtained by immunolocalisation studies *in vivo* and *in vitro*, and by RNAi suppression of the expression on this xylem specific gene in hybrid aspen will be reported.

1. Sterky et al. (1998) Proc Natl Acad Sci U S A 95, 13330
2. Hertzberg, Aspeborg et al (2001) Proc. Natl. Acad. Sci. USA 98: 14732
3. Aspeborg et al (2005) Plant Physiol. 137:983
4. Djerbi et al (2004) Cellulose 11: 301
5. Djerbi et al (2004) Planta, in press

S8.8

THE WAYS AND MEANS OF BOOSTING CELLULOSE PRODUCTION IN TRANSGENIC TREES

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Cellulose is an industrially important commodity. Genetic improvement of cellulose production in commercially important trees and crops will impact global forest and agricultural product industries. With the main goal of improving our current understanding of the process of cellulose biosynthesis in aspen trees, we have focused on cellulose synthases (CesA), sucrose synthases (SuSY) and korrigan cellulases (KOR) that are important for secondary cell wall formation in *Arabidopsis*. We have discovered that three secondary CesAs, one SuSY and one KOR genes from aspen are coordinately upregulated during xylem development and tension wood formation where increase in production of cellulose with higher degree of polymerization, increased crystallinity, and superior microfibril organization is typically observed. Our current objective is to clarify the biological roles of these five genes in regulating cellulose production in aspen trees. Transformation of antisense secondary CesA gene-specific constructs into aspen hindered normal plant development, while positive control transformations yielded normal transgenic aspen trees suggesting that the presence of all 3 secondary CesA genes is essential even for normal morphogenesis and differentiation. The same three gene constructs were used to knock-down orthologous CesA gene expression in tobacco. Although transgenic tobacco plants were obtained at a much lower frequency than the control experiments, they were remarkably dwarf, defective in xylem development, and despite normal flowering did not set any seeds. Simultaneous overexpression of three secondary CesAs from aspen in tobacco and aspen resulted in transgenic plants exhibiting faster growth and increased stem diameter as compared to control plants in both the species. While similar experiments with aspen SuSY and KOR genes are in progress, the antisense inhibition or overexpression of KOR alone in tobacco resulted in plants with defective seed setting. These observations suggest that manipulation of one or more of these cellulose biosynthesis genes may not only alter the cellulose content and quality but could also affect plant growth and reproductive development in transgenic plants. Further molecular and chemical analyses of transgenic tobacco and aspen plants altered in expression of these five genes are currently in progress and results will be presented and discussed.

S8.9

ENGINEERING OF WOOD STRUCTURES

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Prospects for genetic engineering of wood is encouraged by the large natural variation in both the morphology and chemistry of wood and wood fibers induced by environmental and internal cues. Formation of tension wood in angiosperm trees, for example, is perhaps one of the most striking examples of wood plasticity. Wood plasticity is also demonstrated by plant hormones, which can modify most aspects of wood properties when applied to the tree. Although mechanisms underlying these phenomena are by large unknown, cell wall biosynthesis genes should be mediators of the response. We have modified the activity of expansin, pectinmethylesterase and xyloglucan endotransglucosylase/hydrolase in the wood forming tissues of poplar, to evaluate the potential of these primary wall enzymes to modify the morphology of xylem elements. The results show in all cases small but significant effects on vessels and fibers shapes. The presentation will give an overview of the achievements, and discuss future steps in the light of present knowledge.

Centre of Excellence in Wood and Fiber Biology

S8.10

DOWN-REGULATION OF CINNAMOYL-COA-REDUCTASE IN POPLAR: FROM THE LAB TO THE FIELD

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Lignin is an aromatic polymer that needs to be extracted from wood chips for the production of high-quality pulp, a process that consumes large amounts of chemicals and energy. Hence, trees engineered for lower lignin content or modified lignin structure may improve wood quality for pulping. The enzyme cinnamoyl-CoA-reductase (CCR) catalyses the conversion of feruloyl-CoA to coniferaldehyde, the penultimate step in the biosynthesis of the monolignols, the building blocks of lignin. Down-regulation of CCR in poplar effectively reduces lignin quantity, affecting cell wall structure and plant growth. However, transgenic lines could be regenerated with less lignin and apparent normal growth in the greenhouse. These lines have been transferred to field trials for 4 years, to evaluate whether CCR down-regulation in trees could have economic value, and whether growth under field conditions was affected. In parallel, we have analyzed the transcriptome and metabolome of CCR down-regulated poplar using microarrays, LCMS and GCMS. Interestingly, CCR down-regulation affects the transcript level of genes within as well as outside monolignol biosynthesis. These data provide insight into the intricate interactions between metabolic pathways and show how plants adapt, at the molecular level, to defects in the expression of single genes.

WT Poplar

Morreel et al. Plant Physiol 2004 a, b

S8.11

NATURAL VARIATION OF LIGNIN AND THE INFLUENCE OF TRANSGENIC EXPRESSION IN LIGNIN BIOSYNTHESIS IN YOUNG WOOD OF NORWAY SPRUCE

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Lignin content and composition are targets for tree breeding, owing to the environmental gains from reducing the requirements for energy and toxic chemicals when removing lignin from pulps. The aims of this project are to evaluate the natural variation in Norway spruce and to compare this to transgenic material downregulated in lignin biosynthesis. We have compared the lignin content in 1-year-old plants and 9-year-old trees of Norway spruce belonging to the same full-sib families. It was shown that the lignin content according to the modified acetyl bromide method does not vary significantly within or among the different full-sib families either as plants or as young trees. There was however on average 15% higher lignin content and lower standard error for the trees than for the plants. The number of C9-units g^{-1} lignin, analyzed by thioacidolysis, ranges from 516 to 1186 mmol C9-units g^{-1} lignin in plants and from 716 to 953 mmol C9-units g^{-1} lignin in trees, with no significant differences among the families. However, the extent of change in C9-units g^{-1} lignin varied among the families with age. The ratio of *erythro-* and *threo* stereoisomers did not vary significantly among the families. However, the ratio changes differently with age among the families in a similar way as the C9-units g^{-1} lignin. Additionally, the content of *p*-hydroxyphenyl (H) lignin was 75% higher in the trees than in the plants, indicating a higher degree of compression wood in the young trees. Ten sublines of Norway spruce harboring antisense constructs of cinnamoyl CoA reductase (CCR) fused to the maize ubiquitin promotor and the *bar* gene conferring resistance to glufosinate were grown for five years in a greenhouse in randomized blocks together with transformed and untransformed controls. Gene expression was determined by real-time RT-PCR for CCR and seven other genes of monolignol biosynthesis. Sense CCR expression was lower in the antisense CCR transformants than in the control lines. The expression of the other monolignol synthesis genes was significantly correlated to varying degrees with the expression of CCR, with the genes encoding enzymes acting at the end of the reaction pathway close to CCR showing the strongest correlation. Lignin content determined by the acetyl bromide method was significantly lower in the two sublines with the most reduced CCR expression than in the transformed control line. Resistance to glufosinate was maintained over at least four years, indicating stability of transgene expression. Expression levels of the *bar* gene were highly correlated with antisense CCR expression levels.

S8.12

THE IMPACT OF RNAI-MEDIATED SUPPRESSION OF P-COUMAROYLSHIKIMATE 3'-HYDROXYLASE EXPRESSION ON LIGNIN CONTENT AND STRUCTURE IN POPLAR

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Lignin is an integral, complex polymer conferring both structural support and defence to terrestrial plants. From an operational viewpoint, lignin is regarded as an obstacle to effective and efficient processing of wood, and requires harsh chemical treatments, which are costly both economically and environmentally. Several recent studies have employed genetic engineering of plants to enhance our understanding of the inherent mechanism of secondary wall lignification. This study examined the effects of RNA interference (RNAi) of *p*-coumaroylshikimate 3'-hydroxylase (C3'H) on lignin formation in hybrid poplar. C3'H has traditionally been suggested to be responsible for the hydroxylation of the 3 position of the *p*-coumaric acid, yielding caffeic acid; however, recent studies in *Arabidopsis* have provided evidence that the substrate for the enzyme may instead be *p*-coumaroylshikimate and/or *p*-coumaroylquinate, yielding caffeoylshikimate and caffeoylquinate, respectively. Transgenic plants with RNAi-suppressed C3'H were generated, and analysed with respect to transcript levels, growth parameters, chemical composition, and metabolite flux. Expression profiles confirmed the suppression of C3'H mRNA in a number of lines, and two lines were chosen for detailed analysis, including a strong expresser and a weak expresser. In depth plant characterization revealed a significant decrease in lignin content (~50%) with a commensurate increase in carbohydrate content, as well as a significant alteration in lignin monomer composition. In addition, analyses indicated alterations in metabolite levels consistent with a decrease in C3'H activity, providing further insight into the role of the enzyme in the lignin biosynthetic pathway.

S8.13

PECTIN METHYLESTERIFICATION AFFECTS MANY ASPECTS OF WOOD CELL DEVELOPMENT

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Trees and woody plant species accumulate in their secondary walls the most abundant biopolymers on the Earth: cellulose, lignin and xylan. Pectin is a minor component of mature wood, but an important polymer of developing wood cells. The global transcriptome profiling of poplar carbohydrate active enzymes (CAZymes) in a woody model species, Populus, indicated a high expression of pectin modifying genes during wood development. We functionally studied one of pectin methyl esterases, PttPME1, highly expressed in developing wood. Transgenic poplars were obtained with up- and down-regulated expression of PttPME1, which changed the overall PME activity level, pectin content and methylesterification. Transgenic poplars with low PME activity had highly methylesterified pectin and a decreased uronic acid content. This resulted in an augmented radial cell expansion and an increased intrusive tip growth, leading to the long-fiber phenotype. Opposite effects were observed when the PME activity was up-regulated. Unexpectedly, the secondary cell walls were modified when the pectin methylesterification was changed by the PttPME1 expression. The wood of poplars with highly methylesterified pectin had less lignin and xylan and more cellulose and galactan, and the opposite effects were observed when pectin methylesterification was decreased by PttPME1 expression. The transcriptome analysis of transgenic lines revealed some changes in the CAZyme gene expression but no changes in the lignin biosynthetic genes. This suggests that pectin methylesterification pattern might directly affect lignin polymerization in the walls rather than influence the lignin biosynthesis.

S8.14

GROWTH ENHANCEMENT AND WOOD FIBER IMPROVEMENT BY CELL-WALL MODULATING PROTEINS

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Recombinant cellulose-binding domains (CBDs) and endo-glucanases have previously been shown to modulate the elongation of different plant cells *in vitro* and *in-vivo*. A physico-mechanical mechanism was postulated whereby CBD intercalates between cellulose fibers and separates them in a wedge-like action, whereas endo-glucanase promotes cell-wall relaxation catalytically via cleavage of xyloglucan chains and strained amorphous cellulose polymers. This postulate was further supported by additional *in vitro* experiments in which application of recombinant CBD significantly reduced the wet tensile strength of cellulose paper when tested in an Instron Universal Testing Machine. By introducing the *CBD* gene into plants under an elongation specific promoter and a cell wall targeting signal peptide, we were able to express CBD proteins within the cell wall of plant tissue *in-vivo*. In transgenic plants, expression of bacterial CBD (Family 3 CBM) resulted in accelerated plant growth, as demonstrated in tobacco, poplar, potato and more recently in clonal *Eucalyptus* plants. A similar effect was also observed with plant CBM (expansin) in transgenic *A. thaliana*. Modification of wood fibers by CBD and CBD fusion proteins will be discussed.

S8.15p

DRIMYS WINTERI: A POTENTIAL LONG FIBER HARDWOOD FOR PULP PRODUCTION

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Drymis winteri J.R. et G. Forster, known as Canelo, is a Chilean tree that presents a great potential for timber wood and cellulosic pulp production. The species is found in forest without silvicultural management and minimal commercial explotation. The productivity of *D. winteri* reach values from 23 to 33 m3/ha/yr, similar to *Pinus radiata*, the main commercial wood species of Chile. It can be planted in a wide variety of soils specially in high humidity areas, where others species are not able to grow. The species is a transition between angiosperms and gimnosperms, with phenotypic angiosperm characteristics. The morphological structure is similar of softwood, presenting tracheids (fiber length of 1.5 to 4.3 mm) and low wood density (380 to 500 kg/m3). The chemical composition of a 25-year old D. winteri is 40% glucan, 21% polyoses, 31% lignin and 2.5% extractives. Pulps with 43% of classified yield, kappa number 32 and refined at 38ºSR presented tensile index of 94 Nm/g and tear index of 6.2 mN.m2/g. Our analysis indicated that *D. winteri* is a species that, with forest management and if subjected to the same genetic improvment technology applied to the other commercial trees could reach the statuts of an excellent source of fiber for pulp and paper production in Chile.

S8.16p

MODULATING THE EXPRESSION OF THE GENES *UGDH*, *UXS* AND *UGP* IN *NICOTIANA TABACUM* AND THE IMPACT ON THE CHEMICAL COMPOSITION OF CELL WALL POLYSACCHARIDES

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In higher plants the biosynthesis of hemicelluloses is mainly regulated by UDP-D-glucose dehydrogenase (EC 1.1.1.22) which catalyze the first sugar interconversion of UDPG to UDP-D-Glucuronate. An alternate pathway, which involves the oxidation of myo-Inositol, seems to play an important role for UDP-D-glucuronate formation, and the biosynthesis of uronic acids and pentoses. The first irreversible step in the oxidation of myo-Inositol is catalyzed by myo-inositol oxygenase (EC 1.13.99.1), generating D-glucuronate (glucuronic acid), which is phosphorylated by D-glucuronokinase (EC 2.7.1.43) to D-glucuronate 1-P, and immediately transformed to UDP-D-glucuronate by UDP-D-glucuronate pyrophosphorylase (EC 2.7.7.44). UDP-D-glucuronate residues serve as precursors for the synthesis of UDP-D-xylose, UDP-L-arabinose and UDP-D-apiose. There is a demand for UDP-D-glucuronate in all plant tissues and organs at all stages of development, and it seems that the source of UDP sugars for its biosynthesis may come from either Inositol oxidation or UDP-glucose, depending on the tissue. In order to modulate the biosynthesis of the pentosans and uronic acids and the chemical composition of cell wall polysaccharides we cloned the genes *ugdh* (UDP-Glucose dehydrogenase EC 1.1.1.22), *uxs1* UDP-D-Glucuronate decarboxilase (EC 4.1.1.35) and *ugp* UDP-Glucose pirophosphorylase (EC 2.7.7.9), and expressed in *Nicotiana tabacum*. The changes in the composition of cell wall polysaccharides were determined in stems and leaves of tobacco and will be presented.

S8.17p

CHANGING THE HEMICELLULOSE COMPOSITION IN THE CELL WALL OF *NICOTIANA TABACUM* BY ANTISENSE REPRESSION OF MIO-INOSITOL OXIGENASE (EC 1.13.99.1)

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A full-length cDNA fragment was cloned from *Arabidopsis thaliana* seedlings, using RT-PCR, with an open reading frame of 954 pb and a corresponding protein subunit molecular mass of 37kDa. The deduced amino acid sequence of the cDNA showed a high degree of homology with mio-Inosytol oxygenases from other organisms. The cDNA *miox* 2 gene from *Arabidopsis* was cloned and used to obtain transgenic plants producing antisense RNA. Transgenic homozygous tobacco plants with lower myo-Inositol oxygenase expression were obtained with the number of copies varying between 1 and 7. The repression of the endogenous tobacco *miox* gene caused no major impact on plant development, leaf morphology or flowering time. There was however, a statistically significant ($p<0.05$) increase in the arabinan and D-glucuronate content particularly in one transgenic line. These results clearly indicate that the restriction of the myo-Inositol pathway caused no major impact on cell wall polysaccharide biosynthesis.

S8.18p

MOLECULAR CHARACTERIZATION OF A SECONDARY CELL WALL SPECIFIC GENE (*MAXY-1*) OF UNKNOWN FUNCTION IN *POPULUS TREMULA X TREMELOIDES*

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Hybrid aspen, *Populus tremula x tremuloides*, is an excellent tree model for studies of wood development. Expression profiling during wood development have been carried out previously in order to identify genes involved in the secondary cell wall formation in hybrid aspen. In addition to many genes with predictable functions, many genes were identified with previously unknown functions. The gene with the highest level of expression during early secondary cell wall synthesis was denoted *MAXy-1*. Antibodies were raised against a recombinant fragment of the *MAXy-1* protein in rabbits, followed by purification on an affinity column containing a *MAXy-1* protein fragment. The antibodies were used to verify the presence and the size of the *MAXy-1* protein in xylem protein extracts. Subsequent immunolocalisation studies showed the presence of the protein in the periphery of the living part of xylem cells. Detailed data describing the binding specificity *in vitro* and the specific localization of *MAXy-1* in the wood forming tissues of hybrid aspen will be presented.

S8.19p

AN INTEGRATED PARTNERSHIP IN BIOTECHNOLOGIES TO SUPPORT THE *PINUS PINASTER* BREEDING PROGRAM

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Already managing together for 40 years the French *Pinus pinaster* national breeding program, AFOCEL and INRA have concluded in 2004 a partnership agreement coordinating their pre-existing biotechnology research programs on this species. As their most important competitors on the international market, the French maritime pine forest will take full advantage of biotechnology:
- Somatic embryogenesis as a key to vegetative propagation for optimal deployment and improved power of genetic studies (field tests and genetic transformation).
- Cryopreservation to keep plant material in juvenile and safe state in the long term, and at low cost.
- Transgenesis as a tool for genetical and physiological investigations as well as a prospective performance booster.
- Genomics to unravell the genetic factors controlling traits of ecological and economical importance, as well as monitor the genetic diversity of the species.

Since both institutes have extensive collaborations with other organisms, this partnership brings together a complete cluster of specialized teams to contribute to the sustainable management of genetic resources for both accelerated breeding and preservation of traditional forest. This national platform has been setup as a starting point for international projects on the basis of past and ongoing realisations (*) with particular references to other *Pinaster* research activities (Spain, Portugal, Italy and Australia), and pine biotech countries (Canada, USA, New-Zealand, Germany, Sweden). A close collaboration with breeders, wood and paper technologists as well as specialists in non biological aspects of forestry will facilitate the integration of traditional and modern technologies for faster development of locally adapted improved forest, and an optimised management of genetic diversity.

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S8.20p

ANALYSIS OF TRANSGENIC SOMATIC WOOD SECTORS PROVIDES INSIGHTS INTO GENE FUNCTION AND PROMOTER ACTIVITY DURING XYLOGENESIS

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Progress in our understanding of the molecular control of cambial activity and wood formation in trees was in the past hindered by long generation times, high heterozygosity, seasonal dormancy, high labour demands and a range of other factors. As a result, many researchers have turned to alternative model organisms such as *Arabidopsis thaliana* or tobacco. The recent development of *in vitro* and *in vivo* systems for the creation of transgenic somatic wood sectors in trees has made possible the functional analysis of genes involved in xylogenesis directly in the target tissue and within the tree species of interest. By using *Agrobacterium*-mediated transformation, those systems allow for the production of clonally related transformed xylogenic cell clusters which are surrounded by non-transformed control tissue, with superior transformation efficiency and within timeframes of only a few months as opposed to years needed for many conventional approaches. Such methods hold particular promise for the (high throughput) screening and analysis of genes suspected to be involved in wood formation, complementing large-scale gene discovery and sequencing efforts. Here we provide proof of concept by demonstrating how we have successfully used these systems for promoter studies and for the functional analysis of genes involved in wood quality determination in commercially and scientifically important tree species such as poplar and eucalypts.

S8.21p

CHARACTERIZATION AND VARIED EXPRESSION OF A MEMBRANE-BOUND ENDO-1,4-BETA-GLUCANASE IN SPRUCE

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In terrestrial plants, the process of cellulose biosynthesis is as fundamental and important as photosynthesis. Despite its importance, a complete and thorough understanding of the intricacies of its biosynthetic process(es) remains a mystery. Furthermore, cellulose produced in the primary and secondary cell walls of plants differ significantly both in its quantity, as well as quality. Compared to primary plant cell walls, secondary cell walls contain substantially more cellulose of higher degree of polymerization (DP), higher crystallinity and lower microfibril angle. Although the precise causes of such heterogeneity have only recently began to be discovered, molecular genetic data from several sources suggests that at least two structurally and functionally different types of CesAs are involved in cellulose biosynthesis of primary and secondary cell walls. Additionally, a unique membrane-anchored endo-beta-1,4-D glucanase (EGase), KORRIGAN (KOR), has recently been shown to be essential for cellulose biosynthesis in *Arabidopsis*. However, the exact role of KOR in modulating the cellulose biosynthetic process is still unclear. We have characterized and cloned homologous full-length KOR cDNA from spruce (*SpruceKOR*) and *Arabidopsis* (*AtKOR*; At5g49720), and obtained KOR cDNA from aspen (*PtrKOR1*; Joshi et al., 2004). The isolated KOR homologues were employed in complementation studies with *Arabidopsis* kor1 and kor2 mutants in order to test their functionality. Results related to their level of complementation as well as their degree of homology will be presented. *AtKOR* and *PtrKOR* constructs have also been successfully transformed into spruce (*Picea glauca*) trees, and characterized by a variety of molecular, biochemical, histochemical, microscopic and genetic approaches to elucidate the interactions, locations, and role of KOR in modulating cellulose biosynthesis in trees. We hypothesize that altering the expression of these membrane-bound EGases in spruce will affect the amount and ultrastructural properties of the cellulose synthesized.

S8.22p

CDNA CLONING OF *ASPARAGUS OFFICINALIS* HINOKIRESINOL SYNTHASE

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Norlignans are a class of natural phenolic compounds with diphenylpentane (C6-C5-C6) structure found in coniferous trees and some monocotyledonous plants. Because coniferous norlignans are deposited in heartwood, their biosynthesis is of interest in relation to heartwood formation, a metabolic event specific to woody plants. Hinokiresinol is the simplest norlignan and therefore a good target for norlignan biosynthetic studies. The norlignan has two geometrical (*E*)- and (*Z*)-isomers. (*E*)-Hinokiresinol is typically found in conifer heartwood together with other norlignans, in contrast with the distribution of (*Z*)-hinokiresinol in monocotyledonous herbs including *Asparagus officinalis*. Recently, we demonstrated the enzymatic formation of (*Z*)- and (*E*)-hinokiresinols using *A. officinalis*[1] and *Cryptomeria japonica*[2] cell systems, respectively, as enzyme sources. Our next attention was focused on cloning of a gene encoding hinokiresinol synthase. In this study, we purified hinokiresinol synthase [HRS] from elicitor-treated *A. officinalis* cells and cloned two cDNAs encoding *Ao*HRS. When each *Ao*HRS recombinant protein was incubated individually with 4-coumaryl 4-coumarate, (*E*)-hinokiresinol, but not (*Z*)-hinokiresinol, was formed. In sharp contrast, a mixture of the two recombinant proteins catalyzed the formation of (*Z*)-hinokiresinol, the geometrical isomer occurring in *A. officinalis*, from the same substrate. The results indicated that the *Ao*HRS was composed of two subunits. Furthermore, the subunit composition can control the stereochemistry of the product, which is quite interesting from an organic chemical aspect. In conclusion, the present study has demonstrated for the first time cloning of cDNAs encoding *Ao*HRS.

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S8.23p

A NOVEL LIGNAN *O*-METHYLTRANSFERASE CATALYZING A REGIOSELECTIVE METHYLATION OF MATAIRESINOL

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The *O*-methylation catalyzed by *S*-adenosyl-L-methionine dependent *O*-methyltransferases (OMTs) is crucial to determining final product distribution in many plant secondary metabolism including a wide variety of phenylpropanoid biosynthesis. Genes of many plant OMT members, e.g. 5-hydroxyconiferylaldehyde, caffeoyl CoA, flavonoid, stilbene, eugenol, and alkaloid OMTs, have so far been characterized in detail. However, those involved in methylation of lignans have not yet been reported in spite of the wide distribution of methoxylated lignans in plant kingdom and their having important biological activities, such as antitumor, antimitotic, and antiviral properties. Only several studies reported crude enzyme preparations catalyzing *O*-methylation of some lignans such as matairesinol. In the present study, a crude enzyme preparation from safflower (*Carthamus tinctorius*) was found to catalyze regioselective methylation of matairesinol giving rise to arctigenin (4'-*O*-methylmatairesinol), but not isoarctigenin (4-*O*-methylmatairesinol). This is in sharp contrast with the result of *Forsythia intermedia* OMT, which catalyzed non-regioselective methylation of matairesinol to afford both arctigenin and isoarctigenin (Ozawa et al., 1993). Next, based on a PCR-guided strategy with a degenerated primer designed using conserved base sequences of various plant OMT genes, we isolated a novel putative OMT cDNA from a safflower cDNA library. A recombinant protein prepared from the cDNA catalyzed regioselective methylation of matairesinol to give arctigenin, like the plant protein. On the other hand, the recombinant OMT did not catalyze *O*-methylation of phenylpropanoid monomers (caffeic acid, 5-hydroxyferulic acid, caffealdehyde, 5-hydroxyconiferylaldehyde, caffeyl alcohol, and 5-hydroxyconiferyl alcohol) and a flavonoid (apigenin) was found to be a poor substrate. Taken together, these results indicate that the cDNA encodes safflower matairesinol OMT (*Ct*MROMT) which is a novel type of plant OMT specific to lignan methylation. In conclusion, this is the first report of cloning of a cDNA encoding lignan OMT.

S8.24p

C/N METABOLISM IN CONIFERS: FROM EST TO PROTEIN FUNCTION

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N availability in natural soils is a significant factor limiting plant growth and development. Consequently, different metabolic regulations and interactions have evolved to guarantee the strict economy of this essential nutrient during the plant life. The efficiency of N assimilation and partioning is a key component of tree growth and biomass production. Although recent progress has increased our understanding of how inorganic N is assimilated and metabolized in trees, further research is needed to explore the regulatory networks connecting N assimilation and recycling, tree growth and wood production. We are combining the new techniques of functional genomics with classical methods of enzymatic analysis to study the physiological roles of genes/enzymes involved in N recycling and C/N interactions in pine trees. Currently, cDNA and genomic clones for more than 20 genes have been isolated and characterized. The expression of high-amounts of recombinant proteins in bacteria is allowing rapid biochemical studies and antibody production. New adapted methods for the precise localization of mRNAs and proteins in pine cells and tissues are available and their use is providing new insights on how metabolic pathways are organized in different cell types. The isolation and functional characterization of gene promoters is also a critical step in the elucidation of common regulatory mechanisms between C and N metabolism. Finally, the function of selected conifer genes is analyzed in transgenic poplars. We are using a bioinformatic approach to detect nearly full-length cDNAs in EST databases of maritime (*Pinus pinaster* Ait) and Scots (*P. sylvestris*) pines. About 1000 putative clones have been identified and will be completely sequenced. A collection of full-length cDNAs will facilitate protein identification and functional analysis of conifer genes using the above described molecular tools. A general overview of our research programme will be presented.

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S8.25p

THE CELLULOSE SYNTHASE TOOLBOX OF *EUCALYPTUS*: SIX NEW FULL-LENGTH CELLULOSE SYNTHASES ASSOCIATED WITH PRIMARY AND SECONDARY CELL WALL BIOSYNTHESIS

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Vascular plants harbour a family of cellulose synthase catalytic subunit (*CesA*) genes, which encode components of an enzyme complex localized in the cell membrane. Recent studies in moncot and dicot species demonstrated that two groups of *CesA* genes exist, associated with either primary or secondary cell wall biogenesis. We isolated six full-length *CesA* cDNAs from *Eucalyptus grandis* (*EgCesA1* through *6*) and determined their expression patterns in a variety of tissues from an adult tree. The six *EgCesA* genes are predicted to encode proteins of 978 to 1097 amino acid residues, each of which contains all of the significant motifs particular to functional CESA proteins. The predicted proteins share relatively low amino acid homology with each other, ranging from 61% to 70%. Similar CESA proteins from higher plant species exhibit much higher (81% to 90%) identity with the six EgCESAs. Expression analysis utilizing quantitative reverse-transcription polymerase chain reaction (qRT-PCR) indicated that transcripts of *EgCesA1* through *3* were abundant in tissues which are actively laying down secondary cell walls (e.g. xylem), while being very weakly expressed in tissues undergoing mostly primary wall deposition (e.g. unfolding leaves). Expression of *EgCesA4* and *EgCesA5* was increased in tissues rich in rapidly dividing cells undergoing primary wall synthesis, while *EgCesA6* was very weakly expressed in all of the tissues analysed. These results confirm that *Eucalyptus* expresses two contrasting groups of apparently co-regulated *CesA*s involved in either primary or secondary cell wall biosynthesis. The six newly characterised *EgCesA*s represent a significant proportion of the *Eucalyptus* cellulose synthase gene family and further functional analysis of these important genes is now possible.

S8.26p

CLONING, CHARACTERIZATION AND EXPRESSION PROFILING OF TWO *EUCALYPTUS* SUCROSE SYNTHASE GENES

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One of the functions of sucrose synthase (SUSY) is to supply the immediate substrate, UDP-glucose, for cellulose biosynthesis in higher plants. To clone candidate sucrose synthase genes with this function in *Eucalyptus* trees, we applied degenerate PCR, 3' rapid amplification of cDNA ends (RACE), 5'RACE and genome walking. Two full-length cDNAs encoding sucrose synthase genes, *EgSUSY1* and *EgSUSY3*, were isolated from immature xylem tissue of *Eucalyptus grandis*. A putative pseudogene (designated *EgSUSY2*) was detected during genomic analysis of *EgSUSY1*. The *EgSUSY1* cDNA was 2498 bp in length with an open reading frame of 2418 bp encoding 805 amino acids with a predicted molecular mass of 92.3 kDa. The 2528 bp full-length *EgSUSY3* cDNA contained the same length of open reading frame as *EgSUSY1*, but encoded a polypeptide with a predicted molecular mass of 92.8 kDa. Quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) and phylogenetic analysis revealed that *EgSUSY1* and *EgSUSY3* may have functions related to cellulose biosynthesis during secondary cell wall formation in *Eucalyptus*.

S8.27p

OVEREXPRESSION OF *EUCALYPTUS* CINNAMOYL-COA REDUCTASE IN *ARABIDOPSIS* ALTERS LIGNIN CONTENT AND COMPOSITION OF *ARABIDOPSIS* STEMS

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Cinnamoyl-CoA reductase (CCR; EC 1.2.1.44) catalyzes the first step in the phenylpropanoid pathway specifically dedicated to the monolignol biosynthetic branch. A *Eucalyptus* CCR gene, *EUCCR* , was previously isolated from *Eucalyptus gunnii*. We evaluated the effect of overexpression of *EUCCR* on the cell wall chemistry of transgenic *Arabidopsis* plants. The *Eucalyptus* CCR gene was placed under the control of the CaMV 35S promoter and transformed into Col wild-type *Arabidopsis* plants. In T2 overexpressing plants with a single T-DNA insertion, total lignin was increased approximately 5% in inflorescence stems compared with those of control plants. HPLC analysis revealed that total carbohydrate content was decreased approximately 8% in transgenic stems, suggesting the reallocation of carbon from cellulose to lignin. Thioacidolysis analysis indicated that G-lignin subunit content was increased (15%), while S-unit content was decreased (21%) resulting in an overall reduction of the S:G ratio. These results are consistent with down-regulation phenotypes in tobacco and support the use of *Arabidopsis* to model the role of *Eucalyptus* tree genes in xylem cell wall formation.

S9. Abiotic stress: Interaction of trees with the environment

S9.1

USING FUNCTIONAL GENOMICS TO DISSECT DROUGHT RESPONSES IN PINE

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We have generated more than 172,000 expressed sequence tags (ESTs) from the roots of clonal loblolly pine (*Pinus taeda*) trees exposed to a variety of physical (drought, flooding, cold, heat, dark, gravity), chemical (excess aluminum, boron, copper, iron, mercury, salt, paraquat), and nutritional (insufficient calcium, magnesium, micronutrients, nitrogen phosphorus, potassium, sulfur) stresses. Cluster analyses of this sequence collection identified more than 25,000 unique gene sequences, and quantitative analyses of this data identified numerous genes whose expression patterns across the treatment libraries suggest their involvement in specific metabolic responses to the various stress conditions. A first-generation cDNA microarray containing more than 13,500 unique gene products is being used to probe in greater detail the time course and strength of these responses in trees exposed to stress conditions, with initial studies focusing on drought responses. Results from these initial microarray experiments will be discussed with respect to the gene expression data obtained from the cDNA library analyses (electronic northerns). Also to be discussed will be efforts in the upcoming year to produce a second-generation cDNA microarray that encompasses all of the unique genes identified in the gene discovery effort.

S9.2

INSIGHT INTO THE GENETIC CONTROL OF MOLECULAR PLASTICITY: A CASE OF STUDY IN MARITIME PINE

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Genetic diversity and plasticity are recognized has the two main mechanisms of plant adaptation to their environment. One the one hand, if non-neutral genetic variation exists across habitats, it can contribute to local adaptation in a new range of distribution of an introduced population. On the other hand, phenotypic plasticity provides a mechanism by which populations can tolerate wide environmental variation without genetic change. As a mechanism for tolerance, plasticity can evolve due to natural selection given genetic variation in the degree of plasticity. Genetic differences in plasticity can be represented by different norms of reaction evidenced by significant genotype by environment interaction. If phenotypic plasticity has been widely studied, the study of molecular plasticity has still received little attention. The genomic toolkits have been intensively used to discovering variation in gene/protein expression thus identifying many "expressional candidate" genes for many traits of ecological interest. The problem now lies in the screening of the most relevant candidates, and in discovering which molecular variations really matter, i.e. affect phenotypic variations in natural population. To raise this challenge, new screening tools based on information about the landscape of nucleotide variation have been recently developed by population geneticists. Here we show that the study of the genetic control of molecular plasticity can also provide useful information and form the basis for the development of complementary screening tools to identify the most relevant candidate genes among the tons that are usually produced by genomics. Proteins revealed by two-dimensional gel electrophoresis and showing population x environment interaction (in a factorial design crossing two contrasted *Pinus pinaster* ecotypes by two environments for water availability) were identified by tandem mass spectrometry. Given the function of the proteins showing significant interaction we proposed different hypotheses on the molecular strategies develop by each ecotype to cope with drought, one of the most severe limiting factor of tree growth and development.

S9.3

REGULATION OF WOOD GROWTH BY ABA IN DROUGHT STRESSED POPLAR

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The plant hormone abscisic acid (ABA) has a particular role in plant development integrating environmental signals, such as drought and low temperature, with the metabolic and developmental programs of the plant. In trees, ABA acts not only as mediator of environmental stress, but is also thought to participate in seasonal regulation of wood growth activity and wood cell development. To gain more insights into ABA regulation of annual wood growth in poplar and to unravel the specific role of ABA during drought stress we combined different anatomical, physiological and transgenic approaches. Our data reveal distinct intra-annual variations in wood cell development which alter under drought stress conditions. These variations are associated with specific changes in endogenous ABA levels in wood producing cells. In order to identify physiological active pools of ABA and to monitor sites of drought stress action at a histological level we applied a drought and ABA responsive ß-glucuronidase (GUS) reporter system to poplar. The sensitivity of this transgenic reporter system for drought stress has been verified by preliminary observations. In a further approach we generated ABA insensitive poplar plants transgenic for the mutant *ABI1* gene (*abi1*) from *Arabidopsis*. *ABI1* is an essential part of the ABA signal cascade and *abi1* has already been shown to confer ABA insensitivity in annual plants. Thus, *abi1* transgenic poplar will enable us to study ABA dependent processes in a woody plant. First results from both transgenic approaches will be presented.

S9.4

THE POSSIBLE ROLE OF SP1 PROTEIN IN UNRAVELLING SALT STRESS TOLERANCE IN *POPULUS EUPHRATICA* OLIV., A NEW HYDRO-HALOPHYTE MODEL TREE SPECIES

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Most of the actual studies on plant stress tolerance involve typical glycophytes (i.e. *Arabidopsis*), instead of naturally adapted plants (i.e., halophytes) (Vinocur and Altman 2005). Recently, *Populus euphratica*, an extremely salt tolerant species has emerged as a good model system for studying salt stress tolerance (EU Establish project). *Populus euphratica* is a highly salt tolerant tree, (unlike the salt-sensitive *P. tremula* and other *Populus spp.*) growing in desert regions however requiring moderate soil moisture (i.e., an hydro-halophyte). We have studied and cloned *P. euphratica* trees growing in a small forest located in the Avdat Canyon in the Negev Desert in Israel. Physiological, molecular (differential gene expression) and biochemical (protein and metabolite profiling) parameters demonstrated that many different mechanisms might be involved in the acquisition of salt stress tolerance. In this communication we report on the involvement of a possible mechanism for revealing tree stress tolerance: the expression of a stress-related protein named SP1 previously isolated from *P. tremula* trees (Wang et al. 2002). SP1 was differentially expressed in *P. euphratica* trees upon salt stress both under natural conditions (in the forest) and in controlled pot experiment. In situ sub-cellular localization of SP1-related proteins in *P. euphratica* shows differential distribution of the protein throughout the cytosol (control), while under salt stress SP1 is clearly detected all along the plasmalemma, and in nucleus. This may indicate a salt-induced transport of the protein. SP-1 suffers post-translational modification upon stress called sumoylation. SUMO conjugation is involved in cell differentiation, apoptosis, cell cycle and responses to stress by altering protein function through changes in activity or cellular localization (Johnson 2004). In addition, data on salt-induced differential expression of selected metabolites will be presented.

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S9.5

FROM GENES TO FUNCTION: HOW DOES *POPULUS EUPHRATICA* COMPENSATE SALT-INDUCED OSMOTIC STRESS?

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Industrialisation and urbanisation have reduced land and water availability and caused alarming deterioration of soil, water and air quality. Over-exploitation of forests by inhabitants and changes in agricultural practices have further contributed to loss in ecologically and economically viable environments. A major consequence is increasing soil degradation, desertification and salinisation. Among other measures, for restoration of degraded soils highly stress-resistant tree species are needed. We addressed molecular mechanisms of drought and salt tolerance in the genus *Populus*. This genus harbours species with a wide variation in stress tolerance and has a world-wide distribution. Within this genus *P. euphratica* has been chosen for genomic and ecophysiological analyses because it grows in saline and hot environments such as the Taklemakan desert. Salt-exposure leads to acclimatory structural changes such as leaf succulence in *P. euphratica*. The molecular responses involved in these changes have been analysed using POP-EST, an EST-data base and array technology. In contrast to halophytes or the herbaceous model plant *Arabidopsis*, deposition of sodium in the vacuole is of minor importance. Sodium is mainly accumulated in the cell walls and results in dimished Ca-uptake and influences the metabolome, especially organic solutes for osmotic adjustment.

S9.6

FUNCTIONAL AND POPULATION GENOMICS OF COLD ACCLIMATION IN *PICEA SITCHENSIS*

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Adaptation to winter cold by conifers involves extensive physiological remodelling, which likely corresponds to substantial changes in gene expression. Although functional genomics approaches have been used to study the response of *Arabidopsis* to sustained low, above-freezing temperatures ('chilling'), little is known about the large-scale expression changes that occur in overwintering plants during the fall. As such, we have undertaken a transcript profiling study through the fall hardening period in *Picea sitchensis*. Four-year-old seedlings originating from a population at the core of the species' range (Prince Rupert, British Columbia, Canada) were sampled from an outdoor common garden experiment in Vancouver, British Columbia, Canada, for RNA extraction at five time points between August and December, 2004. To determine phenotype, each individual, on each sampling date, was artificially freeze-tested using a non-destructive, quantitative assay for cold injury. Averages of this data at each time-point indicate a progression from minimal cold hardiness (~60% injury at -10°C) in August, to a high level of cold hardiness by early December (~10% injury at -10°C). To elucidate the temporal pattern of cold-induced gene expression, we are using a *Picea* cDNA microarray containing ~22 000 unique sequences. The timing and extent of cold acclimation in Sitka spruce has been shown to vary widely along a latitudinal cline. Therefore, in addition to expression profiling across all time points in the core population, we are comparing early and late time points between the core population and those at the northern and southern limits of the range (Alaska, USA, and northern California, USA, respectively). Our ultimate goal is to understand the molecular genetic basis for the aforementioned population variation. As a step in this direction, we will survey genes that are up regulated during cold acclimation for among- and within-population nucleotide variation, and seek correlations between this variation and observed phenotypes in an association study.

S9.7

CBF TRANSCRIPTION FACTORS ON *EUCALYPTUS* COLD TOLERANCE

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Based on a functional genomic approach of cold response in *Eucalyptus*, our project targets firstly a better understanding of the molecular mechanisms involved in cold acclimation and secondly the identification of reliable molecular markers (SNPs) for MAS using a candidate gene approach. Due to its fast growth and fiber quality, *Eucalyptus* is the most planted hardwood tree, mainly for the paper industry. However, being an evergreen plant without endodormancy, this species is particularly exposed to winter frost in temperate climates. Although it is widely distributed across the world, its cultivation is mostly restricted to southern areas because of its freezing sensitivity. Its survival during a frost strongly depends on both its constitutive tolerance and its acclimation capacity. This additional and transient increase of frost tolerance takes place in response to a progressive exposure to cool but non freezing temperatures that presage the occurrence of winter frost. Cell dehydration, which is the common damage observed at the cell level after cold, drought or salt stress, strongly affects growth and plant development. It is therefore very important to study the mechanisms underlying the water stress tolerance to provide the basis of effective strategies to genetically improve this trait and consequently the plant productivity. Global profiling data on model or commercial species (rice, barley, sugarcane) give an overview of the large transcriptome changes after abiotic stresses (Fowler and Thomashow 2002; Kreps et al. 2002; Nogueira et al. 2003; Ozturk 2002; Rabbani et al. 2003; Seki et al. 2002). They indicate that pools of genes involved in stress response share regulation pathways between salt, drought and cold stress or, in some cases exhibit stimulus specific regulation. In our hands, the isolation of 300 000 ESTs from *Eucalyptus* (3 cDNA libraries), allowed to identify a pool of 2465 cold-regulated ESTs among a random set of 3470 clones. Beyond the description of the main functional categories of these cold-regulated genes, it will be presented here the gene clustering from macroarray expression analysis according to stress response specificity and kinetics, particularly during *Eucalyptus* cold acclimation. It is clearly established that the CBF (CRT/DRE binding Factor) regulation plays a central role in stress tolerance and in particular in the cold acclimation process, through an ABA independent regulation pathway. The multigenic family of the CBF transcription factors isolated from different plant species exhibit specificities in gene regulation and regulon composition. For example, the CBF pathway is identified in the freezing tolerant species like *Arabidopsis*, as well as in the chilling sensitive tomato, but the CBF regulon is much more limited in the latter case (Jaglo et al. 2001). We isolated 2 members of the *Eucalyptus* CBF multigenic family. The comparison between these 2 different genes and the registered sequences from databases, showed the specific conserved domains (AP2 and CBF signatures) within the coding sequence. For the promoter regions, the analysis allowed the prediction of boxes, which are globally equivalent to those from AtCBF but present at a higher frequency in EgCBF. Interestingly the CRT/DRE box, absent in AtCBF, was identified in the EgCBF promoter which suggests a different way of regulation between the 2 species (Shinwari et al. 1998). Quantitative RT-PCR expression analysis of the 2 EgCBF genes showed an induction by cold, and at a lower intensity by salt or ABA, without regulation by osmoticum, which is in agreement with the literature. However, the 2 EgCBF expression profiles differ for the level of cold induction. As expected, the photoperiod influenced the *Eucalyptus* cold acclimation, but interestingly and reported here for the first time, this effect was found to be correlated with EgCBF expression. In addition, and differently from the reported data for AtCBF (Kim et al. 2002), the light (versus

dark) was found to have a negative impact on EgCBF cold regulation. All in all, these data confirm the high conservation of CBF pathway in flowering plants (both woody and herbaceous). However, they also point out interesting differences with previous reports on herbaceous species, suggesting some specificities in CBF regulation and strengthening the hypothesis of a more complex cold response in this tree.

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S9.8

PHYSIOLOGICAL AND MOLECULAR CHANGES IN RNAI MUTANT ASPEN TREES, LACKING THE PSBS PROTEIN

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Plants in nature have to cope with a variety of biotic and abiotic stresses. Many abiotic stresses lead to over-excitation of the photosynthetic apparatus. Non-photochemical quenching (NPQ) is a process that protects photosystem II from damage during over-excitation. A photosystem II subunit named PsbS has been shown to be essential for NPQ (1). We have previously shown the importance of this mechanism with *Arabidopsis thaliana* mutants lacking NPQ (2). Now, we have created several hybrid aspen (*Populus tremuloides x P. tremula*) mutant lines with reduced levels of PsbS RNA and protein, using RNAi technique. The RNA and protein amount correlated well in the different lines and we got reductions of PsbS RNA and protein between 0 and 95 %. As in the *Arabidopsis* PsbS mutants the amount of PsbS protein correlated to the capacity for NPQ. We have characterized these mutant lines physiologically, using for example pulse modulated fluorescence and thermoluminiscence to investigate the photosynthetic properties of these trees. For the collection of leaf samples we grew the different trees in three different conditions: in the greenhouse under a) standard light conditions (150 μE) b) under high light conditions (600 μE) and under natural conditions outside (varying during the day between 400 and 2000 μE). Samples of plants acclimated to each condition were analyzed for their global gene expression using microarray technique with a 15k cDNA chip. While plants grown under standard conditions showed little difference in global gene expression, plants grown under high light and in natural conditions had many genes with a differential expression between the genotypes. Furthermore the physiological response of the perennial tree seems to be different from that of the annual weed *Arabidopsis*, in that the overall light-harvesting antenna is reduced in size in the PsbS less plants and another protein, CP24, which is thought to play a role in NPQ is present in twice the amounts of wild type aspen. These plants will give us new insight in the regulation of light-harvesting in a tree, where good comparison to a similar mutant in *Arabidopsis* is possible.

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S9.9p

ANALYSIS OF GENE EXPRESSION DURING BUD BURST INITIATION IN NORWAY SPRUCE VIA ESTS FROM SUBTRACTED CDNA LIBRARIES

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We have prepared four subtracted cDNA libraries, forward and reverse, representing genes predominantly expressed in a tree from early flushing (EF) and late flushing (LF) families of Norway spruce, during four weeks before bud burst (for both LF and EF) and seven weeks before bud burst for LF (sampled the same date). Two subtracted libraries during four weeks before bud burst for EF and seven weeks before bud burst for LF were analyzed using PCR-Select Differential Screening Kit (Clontech). And two subtracted libraries during four weeks before bud burst had been partially sequenced. Differential screening reveals that in LF at seven weeks before the bud burst considerably upregulated senescence-associated genes, auxin-repressed proteins and several transcripts without similarities in the Database. Partial sequencing has shown, that in the early library, ESTs encoding proteins of the photosynthetic apparatus, energy metabolism, stress (abiotic and biotic) and senescence related proteins were abundant. In the late library, ESTs encoding metallothionein-like and histone proteins as well as transcription factors were more abundant. We used quantitative RT–PCR to study the expression patterns of 25 chosen genes, and observed that the highest levels of activity for most genes were present when plants were still ecodormant. Late flushing is not a result of a simple delay in gene activity, but rather a consequence of an active transcriptional process. The putative role of the studied genes in regulation of bud burst timing is discussed. Among the candidate genes found, the most interesting ones were the DNA-binding factors, water-stress related genes and metallothioneins. Expression patterns of some genes involved in chemical modification of DNA and histones support our suspicion that epigenetic factors may be involved in the timing of bud burst. In the obtained transcriptomes, we were not able to find genes commonly recognized to be involved in dormancy and bud set regulation (PHY, CRY, ABI, etc.) in angiosperm plants.

S9.10p

OZONE-INDUCIBLE CDNAS FROM LEAVES OF EUROPEAN BEECH (*FAGUS SYLVATICA* L.) AND GENE EXPRESSION ANALYSIS OF SECONDARY METABOLISM

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Suppression subtractive hybridization (SSH) was performed to isolate cDNAs representing genes that are differentially expressed in leaves of *Fagus sylvatica* upon ozone exposure. 1248 expressed sequence tags (ESTs) were obtained from 2 subtractive libraries containing early and late, respectively, ozone-responsive genes. Sequences of 1139 clones (91 %) matched to the EBI/NCBI database entries. For 578 clones no putative function could be assigned. Most abundant transcripts were *O*-methyltransferases, representing 7 % of all sequenced clones. ESTs were organized in 12 functional categories according to the MIPS database. Among them 32 % (early) / 16 % (late) were associated with disease and defence, 15/12 % with cell structure, 3/10 % with signal transduction and 7/6 % with transcription. The expression pattern of selected ESTs [ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (rbcS), WRKY-type transcription factor, ultraviolet-B-repressible protein, aquaporin, glutathione *S*-transferase, catalase a, caffeic acid *O*-methyltransferase and pathogenesis-related protein 1 (PR1)] was analysed by quantitative real-time RT-PCR (qRT-PCR) and confirmed increased transcript levels upon ozone treatment of European beech saplings. The ESTs characterized will contribute to a better understanding of forest tree genomics and also to a comparison of ozone-responsive genes in woody and herbaceous plants. In addition a changed ozone-responsive gene expression pattern of the shikimate pathway and phenylpropanoid metabolism will be presented.

S9.11p

INTEGRATING THE GENETICS AND GENOMICS OF DROUGHT RESPONSE IN *POPULUS*

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The availability of the *Populus* genome sequence facilitates the development of links between the physical and genetic maps of mapping populations. We have used this resource to examine the overlap of QTL mapped in response to drought with gene expression data comparing the transcriptional drought response between genotypes at the high and low distribution extremes for drought response within a QTL mapping population. The response of the population grandparents was also examined and compared. The *P. deltoides* and *P. trichocarpa* grandparents were selected from relatively dry and wet regions of the USA respectively and we therefore hypothesised that natural variation in response to drought would segregate in the F_2 population. Contrasting responses to drought were observed across genotypes, including for leaf pigment content, expansion, and abscission. Surprising divergence of the transcriptional response to drought between genotypes at either end of the population drought response distribution was observed with distinct functional groups of genes activated or switched-off in response to drought. A number of drought specific and drought responsive QTL were identified and the co-location of these genetic regions with genes identified as differentially expressed between both the grandparents and the extreme genotypes was examined. It was found, for example, that a QTL for leaf abscission co-located with a CND41 gene, which functions in controlling senescence onset. Such an approach may prove invaluable for informing candidate gene lists for subsequent expression QTL mapping or for examination within a linkage disequilibrium population for fine mapping.

S9.12p

POPLAR SP1 AND *ARABIDOPSIS* ATSP: UNIQUE STRESS-ENHANCED, HOMOOLIGOMERIC, SELF-ASSEMBLED AND EXTREMELY STABLE PROTEINS IN SEARCH FOR A PHYSIOLOGICAL FUNCTION

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SP1 is a unique plant protein isolated from *Populus tremula* that constitutes 1% of the total cell soluble proteins and displays extreme resistance to various harsh conditions (heat, protease, organic solvents and detergents), and is up-regulated in response to stress conditions such as drought and dehydration, osmotic stress, and temperature stress (1). While the physiological functions of SP1 remain unclear, its structural characterization and in vitro biotechnological implications have been studied in considerable detail (2). SP1 is a unique homooligomeric, self-assembled, thermo-stable and protease resistant protein, forming an extremely stable complex and exhibiting chaperon-like activity in vitro. We have produced a seleno-methionine derivative of SP1 enabling the determination of the crystal structure with a 1.8A resolution (2). The SP1 monomer consisting of four alfa-helices and four beta-strands, two monomers join each other to form a dimmer, and six dimmers create the 145kD dodecamer. Using genetic engineering, we were able to bind nanogold particles to the center of the SP1 dodecamer and create gold-SP1-gold-SP1 alternating nano-tubes. To better elucidate the physiological functions of SP1, we utilize the fact that SP1 is a member of a new protein family, having homologues in many plants, including rice, tomato and *Arabidopsis thaliana*. *A. thaliana* proteins were earlier found to share homology with SP1, and we are now studying the function of the At3g17210 gene product, AtSP, which is highly homologous to SP1. Following cloning of the At3g17210 cDNA and recombinant protein production, we showed its thermostability and resistance to protease, as well as its chaperon-like activity in vitro (citrate synthase protection from heat inactivation). The native protein was isolated from *A. thaliana* using antibodies raised against recombinant AtSP, and its identity confirmed by LC-MS. With an attempt to understand the biological functions of *Atsp*, we transformed *A. thaliana* plants with an RNAi vector resulting in several independent lines showing no apparent AtSP protein. Preliminary results show that growth of the RNAi line is considerably reduced. In addition, screening the Koncz T-DNA knockout library we detected a T-DNA insertion line with reduced expression of AtSP protein and which seems more sensitive to salt stress.

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S9.13p

THE DISCOVERY OF A TRANSPORTER INVOLVED IN SOIL AMINO ACID UPTAKE

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It is known that plants have the ability to take up soil amino acids, but the transporter/transporters responsible have this far not been identified. We have been able to isolate one probable candidate gene/transporter involved in the uptake of soil amino acids. Mutated *Arabidopsis thaliana* seeds were selected for reduced uptake of amino acids. Two independent *Arabidopsis* mutants displayed reduced uptake during selection, one EMS plant and a T-DNA knockout line, these were later found to be mutated in the same gene. The knockout gene is annotated as an amino acid transporter. Both mutant lines display reduced uptake levels of amino acids in a short-term depletion experiment and a long term labelling experiment. Mutant plants have reduced biomass when grown on agar with nitrate and a selection of individual amino acids. When grown on agar with nitrate as the sole nitrogen source, the biomass of mutant lines does not differ from wild type, suggesting that endogenous amino acid transport is not affected. Transgenes with different promotor setups will be used to investigate the function of the transporter in more detail.

S9.14p

ISOLATION AND CHARACTERISATION OF *EUCALYPTUS GLOBULUS* C-REPEAT BINDING FACTOR (CBF) GENES

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In forestry, one of the main cultivated species is *Eucalyptus globulus* due to its high productivity in several countries. This species, which originated in Australia, has been planted in Chile in more than 500,000 ha. A common problem in young *E. globulus* plantations is the low tolerance to cold temperatures which produces significant losses to the forestry industry. One of the mechanisms that could be used by *E. globulus* to adapt to low temperatures may be linked to the expression of CBF genes, as in other species. These genes are transcription activators that turn on a cascade of genes which provide plants with resistance to low temperatures and dehydration. To test this hypothesis we have prepared complementary DNA of *E. globulus* from total RNA, extracted from cold-treated seedlings, using Rapid Amplification of cDNA Ends (RACE) technology. A DNA fragment of approximately 650 base pairs was isolated. This fragment encodes for a CBF-like protein with approximately 45% amino acid homology to *Arabidopsis thaliana* CBF-4, one of the four homologous genes described in this species. Expression studies of its transcript will be shown along other experimental results.

S9.15p

CLIMATREE - CLIMATIC CHANGE AND THE DEVELOPMENTAL BIOLOGY OF DORMANCY CYCLING IN FOREST TREES

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Forest trees, like other perennial plants, have evolved mechanisms to anticipate the environmental alterations that characterise seasonal progression. At the end of the growing season they cease development and assume a dormant and freezing tolerant state. In the course of winter they break dormancy, thereby anticipating spring. However, as long as the temperatures remain low freezing tolerance is retained. The synchrony of these events with the seasons adequately prevents freezing damage, but global warming might compromise this synchrony. The World Meteorological Organization (WMO) and the Intergovernmental Panel on Climate Change (IPCC) reported that global warming will not only lead to a higher average temperature, but also to climatic instability. Particularly climatic instability may disrupt the timing of developmental and acclimation-associated events in forest trees, thereby endangering their survival. The CLIMATREE-project investigates how selected forest trees synchronize their activities with the seasons. The project thereby focuses on the perception of photoperiod in the leaves, acclimation of tissues, and the responsiveness of the shoot apical meristem (SAM). As the SAM is the central player in the cycling between dormant and active states it is vital to obtain a deeper insight into its organization and function. We study therefore cellular, molecular and genetic processes that collectively orchestrate cell-cell communication, and thereby the functioning of the SAM as an integrated whole. In addition we investigate how the SAM transitions through dormancy cycles, and toward flowering. Cell-cell communication is addressed by investigating how two complementary ways of signalling, via plasmodesmata and via ligand-receptor interactions, are involved in coordinating the activities of genetically distinct areas in the SAM, and how this is modulated during the seasons. We thereby use seedlings of birch and Norway spruce as model trees in dormancy cycling studies, with parallel studies in *Arabidopsis*.

S9.16p

COLD ACCLIMATION IN *EUCALYPTUS DELEGATENSIS*: EXPRESSION PROFILING IN NATURAL POPULATIONS

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Sensitivity to low temperatures and frost are major factors limiting the climatic range in which commercial eucalypt plantations can be successfully established. Increasing frost tolerance is therefore an important objective of some breeding programs. Similarly, low temperatures influence the natural distribution of species. Patterns of variation in cold tolerance can be used to study evolution in natural populations and provide essential information for forest management practices (e.g. seed transfer guidelines and re-vegetation). Much hope is held for molecular techniques both as tools for studying the genetic basis of cold and frost tolerance and for unravelling underlying physiological pathways. We are examining gene expression in response to low temperature stress in natural populations of *Eucalyptus delegatensis* using a macroarray of 180 candidate genes derived from a 'cold-treated' *Eucalyptus nitens* cDNA subtraction library (including 52 unknown genes). This array is being interrogated by hybridisation with complex cDNA probes derived from *E. delegatensis* families exposed to controlled low temperatures. Cross species hybridisation highlights the utility of heterologous probes for detecting differential gene expression and the analysis of expression profiles over time reveals the progression of transcriptional responses allowing examination of cold response pathways. Comparison of long- and short-term cold acclimation gene expression should provide insights into fundamental differences and similarities between the short-term frost response and long-term cold tolerance, and their relative importance in the evolution of natural populations.

S9.17p

MOLECULAR AND PHYSIOLOGICAL STUDIES OF SALT TOLERANCE IN EUCALYPTS

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Effects of salinity on morphological, physiological and molecular aspects of plant growth have been investigated in numerous studies. In eucalypts, despite their important role in rehabilitation programs of salt-affected areas, current knowledge is limited to the first two aspects. We used *E. camaldulensis* x *E. globulus* hybrid clones of known salt tolerances (Saltgrow, Australia) to investigate molecular mechanisms utilised by these plants in response to salt stress. Effects of salinity on plant physiology and molecular biology were assessed after exposing clones to varying salt (NaCl) concentrations for different periods of time in hydroponic experiments. *Ecg*NaH, a gene fragment with homology to vacuolar Na+/H+ antiporter genes which when overexpressed confer salt tolerance in many plant species, was identified and isolated from one hybrid. Also, fragments of genes known to be involved in nutrient uptake were identified and isolated with particular focus on *Ecg*PT, a gene encoding a Phosphate Transporter. Expression studies using Northern-Blot analysis revealed that *Ecg*NaH was upregulated in the presence of salt in an organ-specific manner, and increased over time when plants continued to be exposed to salt. *Ecg*NaH was differently expressed in different clones, indicating, in combination with growth data, different levels of salt tolerance. Also, the expression of *Ecg*PT was found to be affected by salt, suggesting a direct effect of salinity on phosphate uptake. *Ecg*PT expression was regulated in an organ-specific way, and a time course analysis showed that *Ecg*PT was increasingly upregulated after longer exposure to salt reaching a peak after three weeks of exposure, indicating the plant's adaptation to the lower P status caused by salinity. *Ecg*PT was expressed differently in different clones indicating different degrees of phosphate transporter activation to accommodate salt stress. Molecular data are discussed in relation to growth data and measurements of ion levels in different organs of different clones and under various salt regimes during the course of the hydroponic experiments.

S9.18p

DEFENSE GENES AGAINST PATHOGENS AND INSECTS ARE UP REGULATED BY COPPER STRESS IN *POPULUS DELTOIDES*

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Phytoremediation of heavy metals using poplars is an interesting alternative considering their fast growth, high biomass production, and elevated transpiration rates. However, successful application of this technology depends on the ability of trees for facing both heavy metal stress and occurrence of pathogens and herbivores. In order to increase our understanding about the defensive response of poplars growing under heavy metal stress, a transcriptomic approach was utilized for analyzing the underlying molecular mechanisms. Plants of a *Populus deltoides* clone originating from rooted cuttings were grown in a hydroponical system for four weeks and treated with copper (30 microM and 60 microM) during 12 and 24 h. RNA extracted from roots was analyzed utilizing 4.6 k cDNA arrays (*P. trichocarpa x deltoides* root and leaf ESTs). A significant up regulation of multiple defence genes was observed in the four treatment combinations assessed. These genes encoded Kunitz trypsin inhibitor TI3, pathogenesis-related protein 6 and 10, chitinase I and III, nematode resistance protein Hs1pro-1 and glutathione S-transferase. A second set including genes related to other wound and elicitor inducible proteins was up regulated in specific treatments and interactions. Up regulation of defence response genes in copper stressed poplars could indicate the presence of a common signal transduction pathway and a probable induction of cross-tolerance.

S9.19p

TWO CBF TRANSCRIPTION FACTOR GENES ISOLATED FROM *EUCALYPTUS* DIFFERENTIALLY RESPOND TO ENVIRONMENTAL STRESSES

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Cold, drought and high salinity are the most common environmental stress factors that influence plant growth and development and place major limits on plant productivity in cultivated areas worldwide. These abiotic stresses, all damaging the plant cells through dehydration, induce various biochemical and physiological responses in plants to acquire stress tolerance. In particular, cold acclimation takes place during a progressive exposure to cool non-freezing temperatures and results in a transient increase of cell frost tolerance. Among the genes induced this adaptive response, the CBF "CRT-Binding factor" genes (four have been isolated from *Arabidopsis thaliana*) are considered to be essential in environmental stress response. They encode transcriptional activators that were shown to be early induced by cold and/or dehydration and they activate transcription of target genes directly involved in cellular protection such as COR genes (Cold Regulated). The involvement of CBF genes in abiotic stress tolerance was demonstrated in the literature by modulating expression through genetic engineering in *Arabidopsis* or tobacco. The poster is the first report of the transcriptional regulation of this gene on a woody plant. *Eucalyptus* CBF expression profiles during cold, drought or salt stress are described to evaluate the specificity of CBF environmental stress response. It describes the isolation of 2 CBF members from *E. gunnii* (EgCBF1a and EgCBF1b), very distinct in the sequence in particular in the promoter region and exhibiting a differential expression in response to various abiotic stresses. The real time RT-PCR expression data will be discussed in the light of the promoter predictive cis-acting elements. The features of EgCBF1a and EgCBF1b will be compared to the data from the literature on the other known CBF.

S9.20p

A STUDY OF DORMANCY INDUCTION IN POPLAR USING MICROARRAYS

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Plants respond to unfavourable conditions by stop growing and entering a dormant state. This is an essential adaptation in areas where the environment alters dramatically, i.e. freezing temperatures in northern Europe. The transition from dormant state into an actively growing state has been thoroughly studied at the physiological and anatomical level but not as much at the transcriptional level. External signals as light and temperature and internal signals as sugars and hormones act through different overlapping signalling pathways. To learn more about the pathways regulating dormancy we have studied the transcript profile of two different clones with the help of microarrays. The two clones originated from a F_2-generation derived from a cross between interspecific F_1 hybrids (*P. trichocarpa* x *P. deltoides*). Due to a wide latitudal separation of the parents, the F_2 generation has a segregating variation in dormancy related traits. We chose to study the gene expression of two phenotypically characterized clones from the F_2 that showed big variations of the timing of bud set. The two different clones, which set bud after sixteen and thirty days of short day treatment respectively, were monitored for gene expression differences and similarities for a time period of two months. We found that there is a substantial remodelling of the transcriptome when plants enter dormancy. The gene expression patterns also differ between clones, with significantly higher levels of differential expression in the early responder. The transcriptional changes seem both delayed and dampened in the late responder compared to the early responder, suggesting that the late responder is less sensitive to the dormancy initiation signal.

S9.21p

ADAPTATION TO HIGH SALINITY IN POPLAR INVOLVES CHANGES IN XYLEM ANATOMY AND AUXIN PHYSIOLOGY

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To investigate the physiological basis of salt-adaptation in poplar we compared the effect of salt stress on wood anatomy and auxin physiology of the salt resistant *Populus euphratica* and the salt sensitive *Populus x canescens*. Both poplar species showed decreases in vessel lumina associated with increases in wall strength in response to salt, however, in *P. euphratica* at three-fold higher salt concentrations than in *P. x canescens*. Transgenic *P. x canescens* carrying an auxin sensitive GH3:GUS promoter-reporter construct were used to monitor changes in auxin concentration and distribution on the whole tree level under salt stress complementing direct measurements of auxin. The concentration of free indole-3-acetic acid (IAA) decreased under salt stress in the xylem of both poplar species, but to a larger extent in *P. x canescens* than in *P. euphratica*. Only salt-treated *P. euphratica* exhibited an increase in IAA-conjugates in the xylem. This increase was matched by a significantly higher expression of the auxin-amidohydrolase *PeILL3* in *P. euphratica* xylem compared to *P. x canescens* xylem. For functional analysis the auxin-amidohydrolase from poplar was overexpressed in *Arabidopsis*. Increased sensitivity of the transgenic *Arabidopsis* to IAA-Leu showed that the encoded hydrolase used IAA-Leu as a substrate. These results suggest that poplar can utilize IAA-amidoconjugates in the stem as a source of free auxin to balance the effects of salt stress on auxin physiology.

S9.22p

PROTEOMIC ANALYSIS OF SPANISH BROOM (*SPARTIUM JUNCEUM* L.) ROOTS GROWN IN SLOPE

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Slope is a complex environmental condition, where mechanical stresses affect plant growth and development. Spanish broom (*Spartium junceum*L.) is a Mediterranean widespread legume, highly diffused in slope areas. A proteomic approach was used to investigate the molecular basis of plant root response to slope condition. Protein patterns of *S. junceum* roots grown in a greenhouse in horizontal and slope condition were compared using 2-DE. About 1000 protein spots were detected displaying high reproducibly across an isoelectric focusing range of 4-7. Among these, 141 spots showed a statistically significant ($p<0.001$) change in abundance under stress condition. Based on the analysis of spot quantity and quality and slope/horizontal expression rate, 34 proteins (20 up-regulated and 14 down-regulated in slope condition) were selected for tandem mass spectrometry analysis. The peptides sequences were compared with the sequences of two databases: an ESTs database containing DNA sequences of approximately 550,000 ESTs of seven species belonging to the Fabaceae family, as *S. junceum*, and the SwissProt database. For 27 peptides the function was assigned by homology, while 7 proteins did not show any alignment with the sequences of the two databases. The majority of slope differentially expressed proteins matched to proteins involved in several stress response of many different plant species and in the organization of cell structure.

The data from this investigation is available at http://cbi.labri.fr/outils/protic/ProticDB.php.

S9.23p

CONDENSED TANNINS AND CARBON SEQUESTRATION: A CASE STUDY IN CARBON PARTITIONING AMONG SECONDARY METABOLITE POOLS IN *POPULUS*

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Phenylpropanoid-derived phenolic glycosides (PGs), condensed tannins (CTs), and vascular lignin typically comprise 30 to 60% of leaf dry mass in *Populus* species. These products differ in their function and durability, and thus in their impacts on tree fitness, growth, and long-term sequestration of atmospheric carbon dioxide. We are beginning a three-year project to study control of carbon distribution among these sinks. In the experiment described here, source leaves were wounded in order to examine shifts in phenylpropanoid carbon allocation and partitioning in expanding leaves. A cottonwood line previously found to exhibit large wound-induced shifts in CT concentration was selected. As expected, the initial CT response was largely limited to young apical sink leaves where CT concentration tripled. The duration of CT accumulation increased, so that upon full expansion CT concentration in those leaves was 5 to 6 fold higher than control. Although CTs are not considered to exhibit metabolic turnover, CT concentration in those leaves decreased to levels only slightly higher than control 5 to 6 weeks after wounding. Height growth was negatively impacted during the second and third weeks of CT induction by wounding. RT-PCR analysis of more than 20 flavonoid pathway genes is presented. Continued functional genomics analysis of CT, PG and lignin metabolism should improve our ability to exploit the wide natural variation of these pools for enhanced productivity and global carbon management through biotechnology.

S9.24p

OZONE EFFECTS ON TRANSCRIPTS AND SECONDARY METABOLITES OF EUROPEAN BEECH: A LYSIMETER STUDY UNDER OUTDOOR CONDITIONS

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Tropospheric ozone is a serious world-wide air pollutant known to be a ubiquitous abiotic stressor. A lot of publications exist about ozone effects at physiological and biochemical levels of forest trees. However, the molecular biological level is underrepresented, and most of these data are based on laboratory experiments. Effects of ambient and double-ambient ozone concentrations on selected genes of the shikimate and phenylpropanoid pathway of European beech were continuously monitored in a lysimeter experiment under outdoor conditions over two years. Transcripts were quantified by real-time RT-PCR, and phenolic secondary metabolites were analyzed by RP-HPLC. Only small changes in the expression values (up to a factor of 2) were found during spring and summer. However, at the end of the vegetation period in 2003 a strong up-regulation of dehydroquinate synthase, dehydroquinate dehydratase, 4coumarate:CoA ligase and cinnamyl alcohol dehydrogenase was found. As ozone is known to accelerate leaf loss, senescence processes may be involved in the changed gene expression pattern.

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GENERAL INFORMATION

1. CONFERENCE RELATED

Coffees/lunches/dining

Daily coffees, teas and lunches at the conference, the Sunday evening Welcome Cocktail Function, the Mayoral Gala Reception (Monday evening) and the Gala Farewell Dinner (Thursday evening) are included in the conference fee. Coffees and packet lunches will be served in the Dining room of the Sanlam Conference Centre. With the exception of the Welcome Reception, which will be held at the Sanlam Conference Centre on Sunday (6 November) at 19:00, all evening functions will be hosted off campus. We suggest you bring a sweater/light jacket and comfortable shoes, should you be attending the optional evening programmes on the Tuesday and Wednesday evenings and for the optional visit to De Wildt Cheetah Farm.

Conference Secretariat

The Conference Secretariat will be open every day from 07:00 to 17:00 throughout the duration of the conference. Conference material will be handed out and the name-badge, which is your ticket to all sessions, lunches, coffees etc, should be worn at all conference events. The contact mobile numbers for the Secretariat are as follows:
Sarie Mehl – 083 252 7094
Althea Holworthy – 082 563 0656

Conference Venue

The conference will take place at the Sanlam Conference Centre situated on the campus of the University of Pretoria (a campus map is provided at the back of the abstract booklet). Although it is located a short distance from most of the hotels and guest houses, delegates are advised to make use of the bus transport that has been arranged. Detailed bus schedules will be posted in the foyer of each hotel/guest house.

Internet Access

Wireless connection

Wireless access will be available to all delegates (with wireless-enabled laptop computers) in the SRC room at the Sanlam Conference Centre from 6 – 11 November 2005. You will need to obtain the USERNAME and PASSWORD from the Conference Secretariat on registration. No costs involved.

Via the computer room

E-mail facilities and internet access will be available to all delegates at the Bioinformatics computer lab, 3rd floor, FABI Square Bioinformatics building between 07:00 and 18:00 each day (a total of 24 computers are available). A limited number of access cards to the buildings will be available from the Conference Secretariat. Please collect these cards before going to FABI and return them to the conference secretariat **immediately after use** so that they can be available for other delegates. You will need

to obtain the USERNAME and PASSWORD from the Conference Secretariat on registration. No costs involved.

Presentation Download and Preview

IT personnel and computers will be available in the front of the Sanlam Auditorium during the registration period and during session breaks to assist attendees in downloading and previewing their PowerPoint presentations. PLEASE ensure that your presentation is downloaded onto the presentation server by no later than 17:00 on the day before your presentation.

Posters

All posters should be on display by 18:00 on Sunday evening. Numbered poster stands (0.95 m x 1.7 m maximum poster size) will be available, as well as double-sided Velcro tape. Odd numbered posters should be manned by presenting authors during lunch on Monday and Wednesday. Even numbered posters should be manned on Tuesday and Thursday. All posters should be removed by the end of coffee break (15:30) on Friday afternoon.

Messages

For messages and correspondence to the participants both before and during the Conference, the address and phone number are:

IUFRO Conference, FABI, University of Pretoria, Pretoria, 0002
Fax: + 12 420 3960
Phone: + 12 420 3938
Messages will be pinned up on the noticeboard situated in the foyer of the Sanlam Conference Centre.

No Smoking Policy

All meeting rooms and seated functions will be smoke-free.

Special Assistance

Staff from the Conference Secretariat will be glad to help you with any special needs (i.e. physical, dietary). They will not have a first aid kit with them, as had originally been envisaged, but will have the telephone number of a nearby chemist and will organize that either the medicine required by a delegate be delivered to the conference centre, or that the standby vehicle will take the delegate to the chemist. In serious cases, the standby vehicle will take delegates to the Little Company of Mary's 24-hour medical centre.

Sports Facilities

The university sports centre is situated close to the conference venue. All delegates may use the facilities at their own risk. Details will be available from the Conference secretariat.

Transportation

Bus transportation has been arranged between the hotels/guest houses and the conference venue at the University Campus. Transport has also been arranged to get you to the venues for the social functions.

On the two optional evenings, Tuesday and Wednesday (8 and 9 November), transport has been arranged from all hotels/guest houses to the Brooklyn Mall for the convenience of all attendees who will not be attending the optional programmes. There are shops, cinemas and many restaurants at the Brooklyn Mall. Should you wish to make use of this service, kindly make a booking with the Conference Secretariat by **morning tea time.** Pay the driver directly (R60 return trip per person). The bus schedule for these two evenings is as follows:

HOTELS TO BROOKLYN MALL
19:00 & 20:00 Holiday Inn Hatfield
19:10 & 20:10 Sheraton Hotel
19:15 & 20:15 Court Classique
19:20 & 20:20 Courtyard Hotel
19:25 & 20:25 Hatfield Lodge
19:30 & 20:30 Hatfield Manor
19:45 & 20:45 Arriving Brooklyn Mall

BROOKLYN MALL TO HOTELS
21:30 & 22:30 Depart Brooklyn Mall

The staff at your hotel/guest house and the Conference Secretariat will assist you should you need transport to other venues. Attendees are encouraged to make use of the conference transport rather than other forms of public transport. We advise delegates not to walk in the area after dark. Transport tickets for each and every trip, which has been paid for as part of the delegate fees or for the optional evenings, need to be handed to the vehicle driver when embarking. The tickets will be in the delegate's pouch.

Other travel arrangements

Any enquiries regarding additional post conference tours, or changing of air tickets can be discussed with the Conference Secretariat, who will handle any requirements on your behalf. They will still be able to take bookings for any tour the delegates might request. Full details are given in the Social Programme.

Mhondoro is fully booked for the weekend (12 – 14 Nov) but delegates will be placed in similar lodges for the same price in Welgevonden. These are Shidzidzi and Nungubane Game Lodges. They can accommodate a total of 20 guests in intimate elegance offering big 5 game viewing. The price is R5900 per person sharing which includes transport to and from delegate's hotel in Pretoria.

The Cheetah tour on Wednesday afternoon is fully booked. However, the Conference Secretariat can offer a game drive tour at the Premier Game farm, just outside Cullinan. One problem is that the game viewing might not be so good if it is a hot day. Summer time tours are usually at 5pm, but because delegates must be back in Pretoria by 17h30 in time for the trip to the Voortrekker Monument, the tour will take place during the

afternoon, starting at 14h30. The Premier Game farm has all game except lion, buffalo and elephant. See website www.premiergametours.com.

2. GENERAL TRAVEL TIPS (Pretoria and the rest of the country)

Banks and Bureaux de Change

All major cities and banks have Bureau de Change offices. International banks have branches in the major cities. In the countryside, most of the banks in the towns should accept travellers' cheques. There are ATM facilities in most towns and cities. These are usually found at the banks or in the shopping malls and at filling stations. Banking hours are weekdays from 09:00 to 15:30 and Saturdays from 09:00 to 11:00. Certain banks and foreign exchange outlets at Menlyn Shopping Centre provide extended operating hours.

Climate

South Africa has a wonderful climate with more than 300 sunshine days on average per year. The Western Cape Area has a Mediterranean climate with warm, dry summers and cold, wet winters. The northern part of the country is hot in summer, with summer rains, and mild in winter. The heat of a summer's day is often relieved by an afternoon or evening thunderstorm. In November the temperature in Pretoria can range from 30 degrees Celcius at midday to an average night temperature of 12 – 15 degrees Celcius. Showers can be expected as Pretoria is in the summer rainfall area of South Africa. We suggest that you bring a warm sweater for chilly evenings and a raincoat/umbrella for the rainy days. The coastal areas of KwaZulu-Natal and eastern Mpumalanga provinces are subtropical and humid. Snowfalls occur in winter (June/July) in the Drakensberg regions and Lesotho. For detailed information on the weather in South Africa, phone the South African Weather Bureau at 082 162.

Drugs and Alcohol

There are strict drug and alcohol laws in South Africa. No person under the age of 18 may buy or consume alcohol in public. Alcohol consumption in public is restricted to bars and restaurants. All habit-forming and recreational drugs are banned. Take care not to accept drugs or drinks from strangers.

Electrical current

The standard power supply is 220/230 volts AC throughout South Africa, with the exception of Pretoria (230V) and Port Elizabeth (200/250V). Adaptors for electrical appliances are widely available in major towns and cities.

Languages

With a population of more than 40 million inhabitants, there are 11 official languages: Afrikaans, English, Ndebele, North Sotho, South Sotho, Swati, Tsonga, Tswana, Venda, Xhosa and Zulu. English is widely spoken and understood throughout the country, with the exception of the deep rural areas. All official signs and road signposts are in English.

Money and Credit Cards

The currency is the South African Rand. There are 100 cents to the Rand. Banknotes are in denominations of R200, R100, R50, R20 and R10. Silver coins are in denominations of R5, R2 and R1, and brass coins in denominations of 50, 20, 10, 5, 2 and 1 cents. Most establishments accept any of the following credit cards: Visa, Master, American Express and Diner's Club.

Postal and Telecommunications Services

Most post offices operate from 08:30 to 16:00 on weekdays and on Saturdays from 08:00 to 12:00. Telephone directories provide full details of international dialing codes. Cellular or mobile phones can be rented at all international airports or as part of a car rental package from any of the three cellular network companies.

Public Safety and Security

Take the same basic personal safety precautions as you would in any country. Never willingly reveal to any stranger that you are a tourist. Avoid displaying expensive jewellery, cameras and other valuables. It is definitely not advisable to carry large sums of money on you. Carry money, credit cards, passports and travel documents discreetly and out of reach of pickpockets and bag snatchers. Make photocopies of all your documents and leave them in a safe place. If you are using a private vehicle, always drive with the doors locked and the windows closed. Lock any valuables in the boot (trunk) of the vehicle – do not leave anything in view on any of the car seats. If at any time you fear for your safety, call South Africa's national emergency number: 10111.

Smoking

It is an offence to smoke in public areas. Check with all accommodation establishments whether smoking is allowed in the accommodation areas.

Time

The time in South Africa in summer is two hours ahead of Greenwich Mean Time.

Tipping

For most services (if the service standard is satisfactory) a 10% tip is usual. Most restaurants do not include a service charge in the bill, so it is customary to tip the waiter directly or add the tip to the final account.

Useful Telephone Numbers

International enquiries:	0903 9 (24-hour service)
Directory enquiries (national):	1023
Rescue service aviation:	083 1999
Police:	10 111
Difficulty with emergency services:	1022
Time:	1026
Any emergency from a cellular phone:	112

VAT (Value added tax)

This is currently set at 14% and is usually included in the retail prices displayed on most goods and services. Visitors to South Africa may claim refunds on VAT paid on goods to be taken out of South Africa on departure. VAT refund administration offices are found at all the major international departure terminals. To claim VAT, the original VAT invoice document is required. Note that services rendered or goods consumed in South Africa do not qualify for a VAT refund.

Weights and Measures

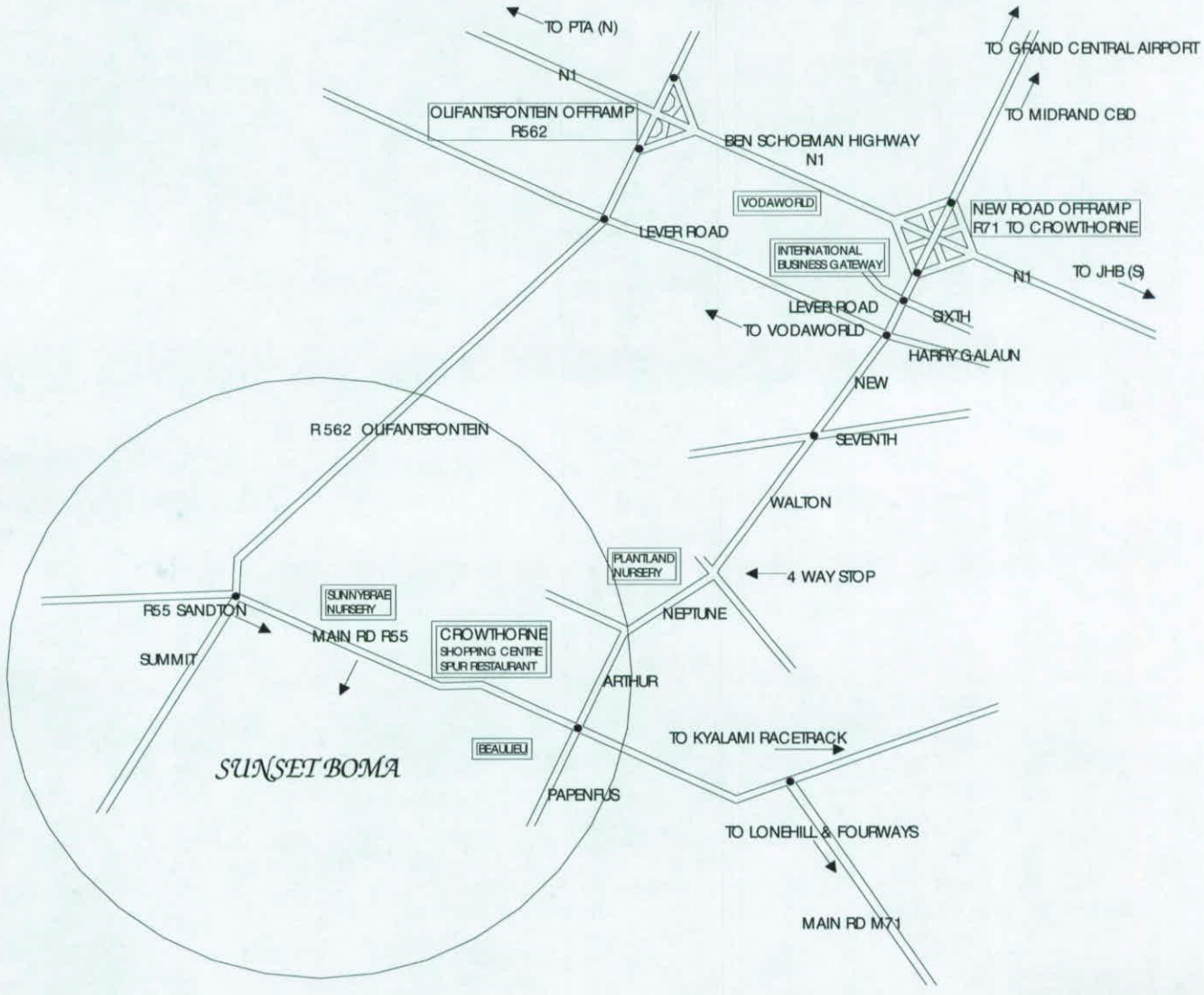
South Africa uses the metric system. Road signs therefore indicate distance in kilometers.

DIRECTIONS TO SUNSET BOMA

(Tuesday 8 November 2005)

79 Main Road, Blue Hills, Midrand
Telephone : (+27 11) 702 2215 Cellular : 082 492 3586
Facsimile : (+27 11) 702 2217 E Mail sunsetboma@iafrica.com

From Pretoria : Travelling on the Pta/Jhb N1 highway, take the Olifantsfontein off ramp. At the traffic lights turn left crossing back over the highway. Continue on Olifantsfontein road for 4 kilometres, until you reach the major traffic light intersection of Olifantsfontein Road and Main Road. Turn Left into Main Road and proceed for approx. 1 kilometre. Sunset Boma will be on your right hand side, opposite the Sunnybrae Nursery and near the 2 cellular towers.



From Johannesburg : Travelling on the Jhb/Pta N1 highway, take the New Road off ramp and turn left onto New Road. Follow New Road, which becomes Walton, then Neptune and finally Arthur Road (approx. 3 kilometres in total). At the traffic lights, the Crowthorne Shopping Centre (Spur Steakhouse) will be on your right. Turn right into Main Road and continue for approximately 2 kilometres. Sunset Boma will be on your left hand side, opposite Sunnybrae Nursery and near the 2 cellular towers.

DIRECTIONS TO PRETORIA COUNTRY CLUB

(Thursday 10 November 2005)

N1 from Jhb

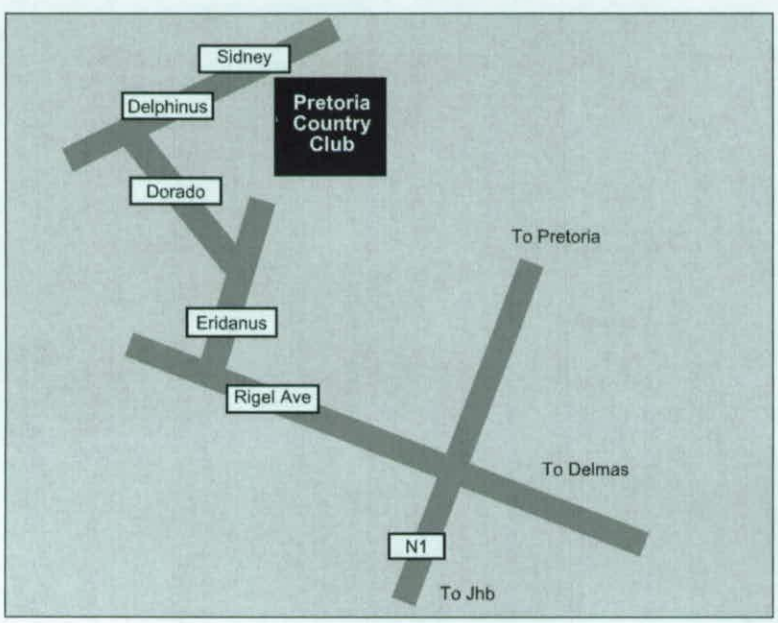
Take Rigel Avenue off ramp, turn left at the robot.
Continue along Rigel Avenue for +/- 5 km.
Turn right at Aries Avenue.
Follow the road around a bend to the left when it becomes Sydney Avenue.
Entrance to Pretoria Country Club, turn right just before speedbump.

N14 from Jhb

Take Eeufees off ramp, turn right at the robot.
Go underneath Bridge and take first road left.
Keep on right side of road, going into Fountain Circle.
Following Brooklyn sign, leave circle at 12 o'clock into George Storar Drive.
Continue along George Storar Drive to the intersection with Queen Wilhelmina Street.
Turn right into Queen Wilhelmina Street.
Follow Queen Wilhelmina Street up the hill until it meets Main Street.
Turn left into Main Street.
Turn right into Sydney Avenue.
Entrance to Pretoria Country Club on your left.

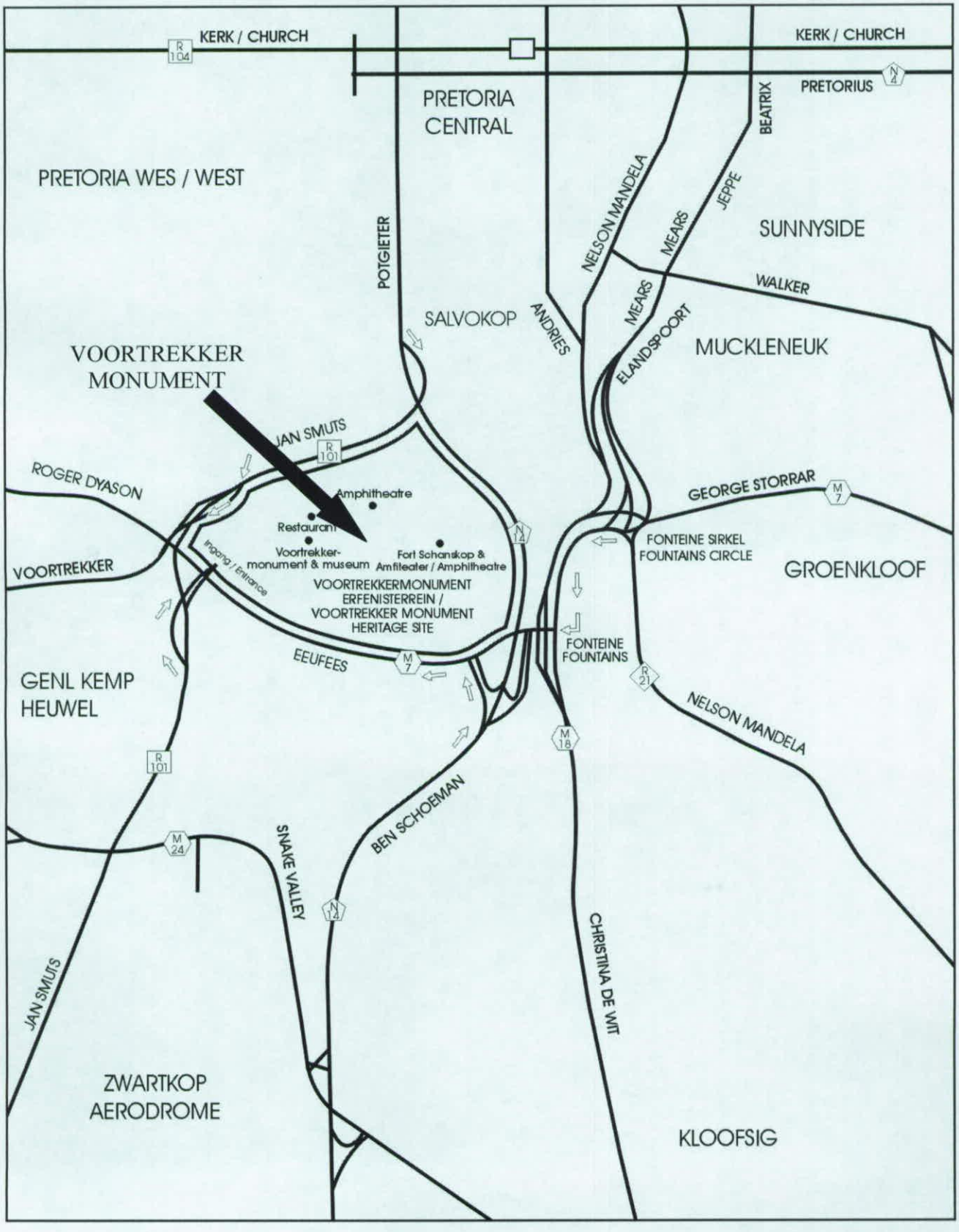
Lynnwood Road from City Centre Pta

Follow Lynnwood Road from Pretoria City Centre in an eastern direction.
Go over Duncan Street, 2nd robot turn right into Brooklyn Road.
Follow Brooklyn Road, 3rd robot turn right into Albert Street.
Over two speed bumps, turn left into Sydney Street.
Over one speed bump, turn left into Pretoria Country Club.



DIRECTIONS TO VOORTREKKER MONUMENT

(Wednesday 9 November 2005)



CHARACTERIZATION AND VARIED EXPRESSION OF A MEMBRANE-BOUND 1,4-BETA-GLUCANASE IN WHITE SPRUCE

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INTRODUCTION

In plants, the process of cellulose biosynthesis is as fundamental and important as photosynthesis. The modulation of cellulose biosynthesis influences many aspects of plant growth and development, including cell division and expansion, plant morphogenesis, and response to environmental cues. Despite its importance, a complete and thorough understanding of the intricacies of its biosynthetic process(es) is still relatively unknown. Elucidating the biosynthetic mechanism of this polymer is pivotal to future experiments aimed at augmenting cellulose production in plants. If attempts to improve cellulose production in commercially important plants are successful, they will deliver enormous benefits to global agriculture and forest products industries. Recently, a unique membrane-anchored endo-beta-1,4-D glucanase (EGase), KORRIGAN (KOR), has been shown to be essential for cellulose biosynthesis in both the primary and secondary cell walls in Arabidopsis *[1]*. Currently, three possible functions have been proposed for KOR proteins during cellulose biosynthesis: 1) removal of a precursor primer molecule from the elongating cellulose chain *[2]*, 2) facilitating cellulose microfibril formation by excising the amorphous cellulose chains, and thus performing a proofreading/editing function that may ultimately increase the crystallinity of cellulose *[3]*, and 3) regulating the DP of cellulose during or subsequent to microfibril assembly *[3]*.

OBJECTIVES

- To generate white spruce (*Picea glauca*) expressing KOR from either *Arabidopsis* (AtKOR) or from aspen (PtrKOR) under the control of a constitutive (2×35S) *[4]* promoter.
- To determine the level of functional conservation between the KOR from white spruce (PgKOR) and AtKOR.

METHODS

- KOR proteins from three different plant species were compared (Figure 1).
- Transgenic white spruce (*P. glauca*) embryos expressing AtKOR and PtrKOR were created using *Agrobacterium*-mediated transformation.
- Successful translines were confirmed using polymerase chain reaction screening of genomic DNA, while the relative level of transgene expression was determined using real time PCR (Figure 2).
- Transgenic and wild-type embryos were grown on semi-solid media in tissue culture in the dark and then transferred to maturation media under 16h days (Figure 3).
- Two KOR mutant lines (kor1-1 and kor1-2) were transformed with the KOR gene from white spruce (Figure 4).

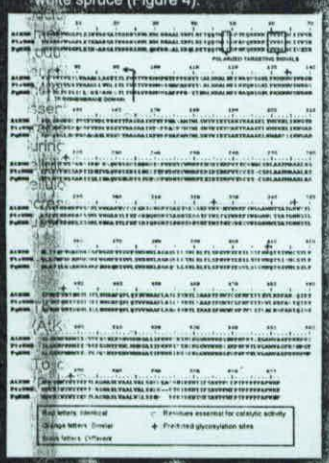

Figure 1. Comparison of three plant KOR protein sequences

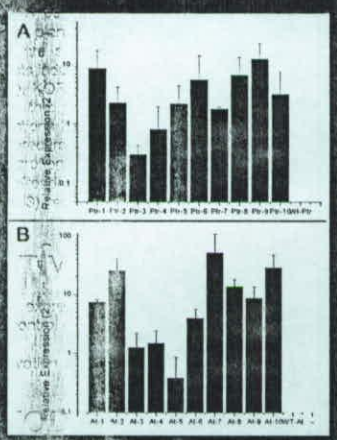

Figure 2. Relative expression of the aspen KOR (A) and the *Arabidopsis* KOR (B) in white spruce embryos (error bars = St. Dev).

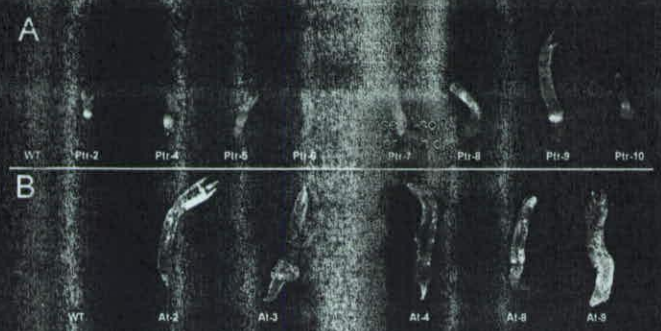

Figure 3. Eight week old white spruce plantlets over-expressing the aspen KOR (A) and *Arabidopsis* KOR (B)

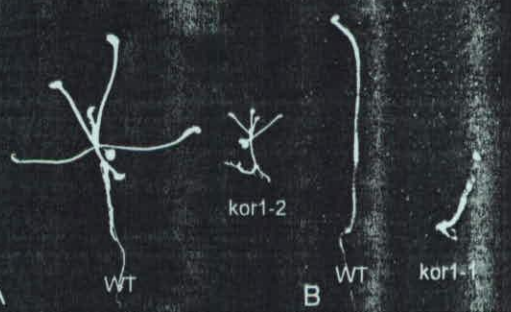

Figure 4. Fifteen day-old kor1-2 (A) and kor1-1 (B) *Arabidopsis* mutants. kor1-2 mutants were grown in the dark.

RESULTS

PROTEIN SEQUENCE ALLIGNMENT

- Alignment of KOR protein sequences illustrates evidence of high similarity between all three proteins (PgKOR:AtKOR = 73%, PgKOR:PtrKOR = 78%, AtKOR:PtrKOR = 83%), and also reveals identical polarized targeting signals (Figure 1).

TRANSFORMANT SCREENING

- PCR screening of genomic DNA demonstrates the establishment of several transgenic lines of white spruce harbouring a foreign KOR gene (both aspen and Arabidopsis).
- Real time PCR of the transgenic lines clearly indicates significant over-expression of the KOR transgenes in the embryonal spruce tissue. The plants transformed with the AtKOR appear to have a higher relative expression compared to the PtrKOR transgenic lines (Figure 2).

PHENOTYPIC OBSERVATION

- Following 8 weeks of growth, distinct phenotypes are visible among the transformed spruce lines (Figure 3).
- The PtrKOR plantlets generally appear to display a "swollen" phenotype, while the AtKOR plantlets show significant increases in elongation and expansion, compared to the corresponding wild type controls of identical age.

ARABIDOPSIS COMPLEMENTATION

- The kor1-2 mutant and the dark grown kor1-1 mutant show dramatic difference in height when compared to the wild type, as previously published *[5,6]* (Figure 4).

CONCLUSIONS

- White spruce transgenic lines harbouring AtKOR or PtrKOR under the expressional control of the 2×35S promoter have successfully been established.
- Select translines show significantly elevated expression of the foreign KOR gene.
- Phenotypic analyses of the transformed spruce lines suggests that the higher expression of the AtKOR transgene, compared to the PtrKOR, directly effects the plantlet growth characteristics.
- Extensive analyses of cellulose content and ultra-structural characteristics are currently underway on the stems of the white spruce, and further transformations will be conducted using RNA inference (RNAi) and epitote tagged constructs in the near future.
- *Arabidopsis* mutants (kor 1-1 and kor 1-2) have been successfully complemented with the PgKOR construct, and in depth analyses are forthcoming.

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TWO CBF TRANSCRIPTION FACTOR GENES ISOLATED FROM EUCALYPTUS DIFFERENTIALLY RESPOND TO ENVIRONMENTAL STRESSES

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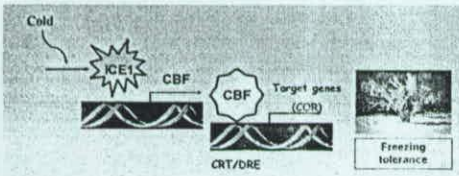
Introduction

Due to fast growth, good fiber properties and low requirement for soil quality, Eucalyptus is one of the world's most economically important hardwood species. Its small genome (above 580 Mbp) makes this tree particularly adapted for genomics studies as well as poplar. As an evergreen perennian, without endodormancy, Eucalyptus is entirely and commonly exposed to frost and survival of sensitive organs such as leaves strongly depends on cell cold tolerance. Therefore, molecular mechanisms of cold response are likely to exhibit more complexity than for most of the plant species. In order to better understand these mechanisms and benefiting from previous biochemical and biophysical data as well as available tools (cell supension cultures, Eucalyptus genetic transformation procedures), we are developing a functional genomics project on this species. We already isolated pools of cold regulated genes and among them, we first focused on a CBF-like gene, due to the known prominent role of CBF pathway in cold acclimation.



This poster presents the regulation by cold of two CBF1 isolated from *Eucalyptus gunnii*, Egu*CBF*1a (DQ241820) and Egu*CBF*1b (DQ241821). Temperature shocks and acclimation program were applied and the transcript level of the 2 Egu*CBF*1 genes was quantified by real time RT-PCR. The effect of photoperiod during the acclimation program was also studied.

Results

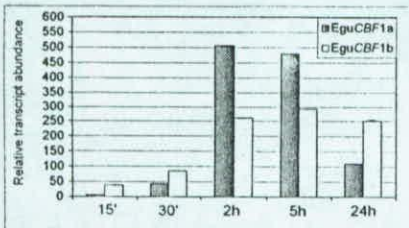


Fig 1: Time course of Egu*CBF*1 gene expression at 4°C under dark culture. The relative abundance of Egu*CBF*1a/b transcript was quantified using real time RT-PCR.

- Egu*CBF*1a and b are **quickly** and **strongly induced by low temperature** but with a different level.
- At 24h the induction of Egu*CBF*1b is still strong, while it declines for Egu*CBF*1a

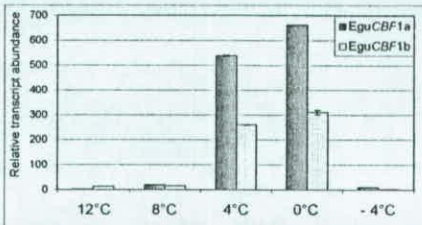


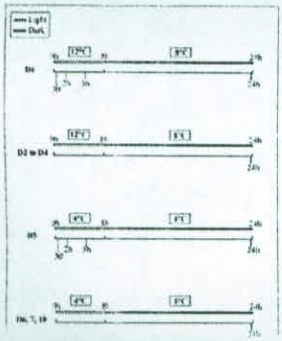
Fig 2 : Effect of cold shock intensity on the expression of Egu*CBF*1 transcription rate. Plantlets were exposed for 2 hours in the dark to each indicated temperature and the relative abundance of Egu*CBF*1a/b transcript was quantified using real time RT-PCR.

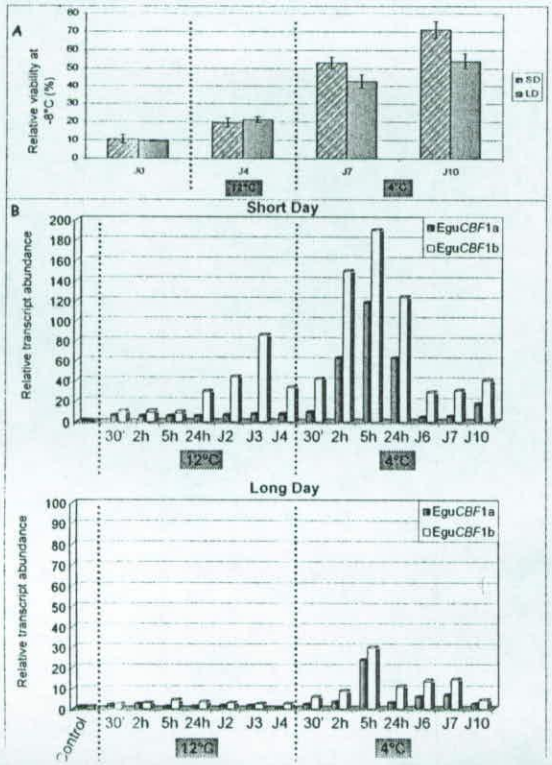
- Decreasing positive temperature results in higher accumulation of Egu*CBF*1 transcript with a maximum at 0°C.
- Below 8°C transcript acumulation is higher for Egu*CBF*1a than for Egu*CBF*1b, while at 12°C it is the opposite.

The 2 Egu*CBF*1 genes exhibit a differential and complementary regulation by cold, Egu*CBF*1a being more transiently induced and responding better to lower temperatures, Egu*CBF*1b has a longer time course.

Material and methods

Three plantlets previously grown for three days at the appropriate photoperiod were used as a control and then transferred under chilling conditions for four days at 12°C-day / 8°C-night (D4), then six days at 4°C (D5 to D10). The freezing tolerance was evaluated on D4,D7 and D10, by ion leakage measurements on leaf discs frozen to -6°C (A). On the same plants, total RNA was extracted from Eucalyptus leaves randomly harvested at different times along the cold culture program (fig 3). The transcript accumulation of Egu*CBF*1b during the short day (SD) or the long day length (LD) acclimation program of plantlets was quantified using real-time RT-PCR (B).





- Short Day photoperiod positively affects the freezing tolerance.
- Short Day photoperiod positively affects the expression of the 2 Egu*CBF*1 genes.

The maximum gene induction rate for the 2 genes is observed in short-day conditions during the 4°C period.

Always more highly induced than EguCBF1a, Egu*CBF*1b is the only gene significantly responding to 12°C.

The 2 Egu*CBF*1 genes are likely to be involved in Eucalyptus freezing tolerance but with different regulation profiles.

Conclusions

The expression patterns of the 2 Egu*CBF*1 genes evidenced some complementarities in the regulation: the Egu*CBF*1a exhibits an efficient short-term response to cold shock conditions whereas the Egu*CBF*1b shows a better and longer response to more moderate or progressive temperature changes. In order to better evaluate

ISOLATION AND EXPRESSIONAL ANALYSIS OF 1995 COLD-REGULATED ESTs FROM EUCALYPTUS

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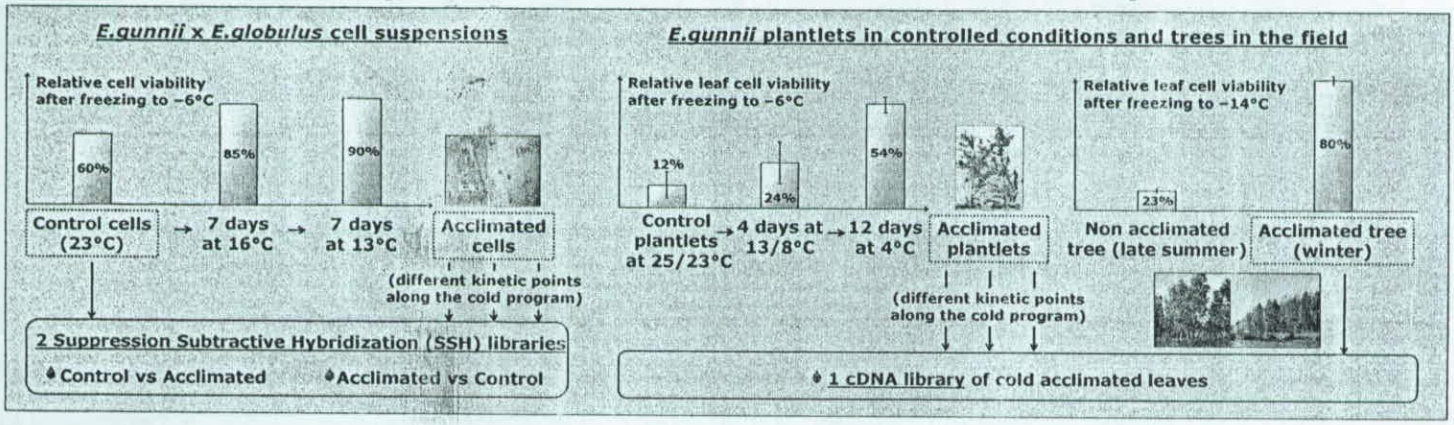
Due to fast growth and fiber quality, *Eucalyptus* is very important for the paper industry. However, being an evergreen woody plant without endodormancy, this species is particularly exposed to cold. Although widely distributed across the world, its extension is mostly restricted to southern areas by freezing sensitivity. Its survival during a frost strongly depends on its constitutive tolerance at the cell level and also on its acclimation capacity.

Based on a functional genomic approach of cold response in *Eucalyptus*, the project is targeting a double objective :

- Better understanding of molecular mechanisms involved in cold acclimation of *Eucalyptus* including the protective response and its regulation.
- Identification of reliable molecular markers (SNPs) for cold tolerance for MAS using a candidate gene approach.

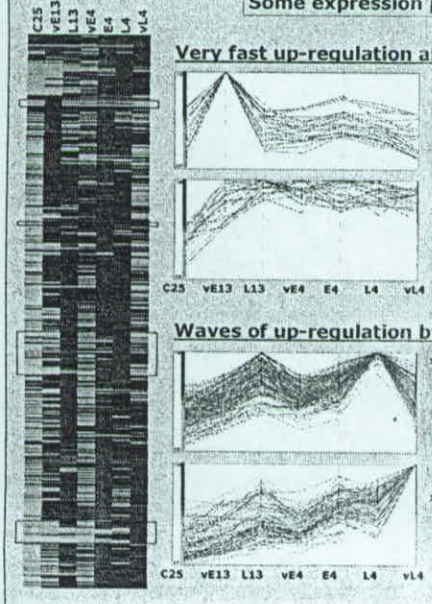
⇒ Isolation of cold-regulated genes



⇒ Expressional analysis of 1995 ESTs

3899 ESTs from the 3 combined libraries were PCR amplified and spotted in duplicates on nylon macro-array filters. The filters were hybridized with ^{33}P-labelled cDNA from leaves of *E.gunnii* plantlets harvested at different kinetic points along the cold program described above. Signal intensities were quantified using ArrayGauge software and normalized using signals from water, empty vector PCR product and control genes that are not affected by cold as assessed by real time RT-PCR. ESTs that didn't show signal reproducibility were cancelled from the analysis. Finally, the normalized expression levels of 1995 ESTs were studied along the cold acclimation program.

Some expression profiles of cold-regulated genes



Very fast up-regulation after exposure to cold

- *Transiently induced
  - Early light induced
  - ERD15 (early responsive to dehydration)
  - Photosynthesis: chlorophyll a/b binding, rubisco, photosystem I
- *Induction maintained along the cold program
  - Detoxication: glutathione transferase, metallothionein

Waves of up-regulation by both temperature switches

- *Maximum when acclimation begins to be significant (after 6 days at 4°C)
  - LEA (late embryogenesis abundant)
  - Proteolysis: ubiquitin-conjugating enzyme E2, arginase, β2 subunit of 20S proteasome
- *Maximum at the end of acclimation
  - Dehydrin
  - β 1-3 glucanase (potential antifreeze)

Hierarchical clustering of cold-regulated genes. For each cDNA, expression level at a given kinetic point was compared to the highest expression level for this cDNA among all kinetic points. Color scale goes from green (lowest level) to red (highest level). Studied kinetic points are given. C25: control at 25°C; vE13: very early (15', 30' and 2h) at 13°C; L13: late (2, 3 and 4 days) at 13°C; vE4: very early (15', 30' and 2h) at 4°C; E4: early (5 and 10h) at 4°C; L4: late (2, 3 and 6 days) at 4°C; vL4: very late (9 and 12 days) at 4°C.

A powerful tool to identify candidate genes for cold tolerance in *Eucalyptus*

Few global transcriptome analyses are reported on woody species with regard to cold acclimation and they mainly concern dormancy. Here, the isolation and expressional analysis of cold-regulated genes from *Eucalyptus* is presented.

- Enrichment of genes with key role in cold acclimation and stress tolerance: detoxication enzymes (metallothionein), LEA, CBF regulon (galactinol synthase), potential antifreeze...
- Over-representation of dehydrins. These proteins supposed to play a major role in tree cold tolerance were gradually up-regulated along the cold acclimation program and showed the highest rates of induction among all ESTs in the libraries.
- Up-regulation of genes involved in sugar or lipid metabolism, in proteolysis...
- Good representation of transcription factors.

⇒ Candidates for cold tolerance in *Eucalyptus*?

The expression profiles for some of these genes will be further studied by real time RT-PCR (specificity, relation with the degree of tolerance for different genotypes...) to identify the best candidates for marker assisted selection.

- 9% ESTs encode proteins of unknown function and 28% share no homology with sequences in the public databases

⇒ Potential source of new genes involved in cold tolerance. They will also be considered for candidate identification.

CBD technologies

TRANSFORMATION OF COMMERCIAL EUCALYPTUS CLONES

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ABSTRACT

We are developing unique technology for crop and fiber enhancement mediated by cell-wall modification genes. The deployment of our technology for forest plantations is being realized via transformation of clonal *Eucalyptus*. The transformation of non-juvenile plant material is a challenging task. The *Eucalyptus* explants of clonal material maintain mature properties of their mother trees. In addition, the genetic variation between the different clones of the same species dictates development of a wide range of protocols for different clones. We have developed efficient tissue culture methods for the transformation and regeneration of clonal *Eucalyptus*. We have succeeded in transforming a number of commercial *Eucalyptus* species, including elite *E. camaldulensis* , *E. grandis* and several commercially important *E. grandis* hybrid clones. We have established regional collaborations with leading forestry companies to develop high-performing *Eucalyptus* germplasm for the plantation forest industry. Our first field test with transgenic *Eucalyptus* was planted in 2003 in Israel. This experiment includes several hundred trees expressing the cbd & cel1 genes under different promoters. Additional experiments are underway in commercial territories.

Clonal plant in tissue culture

First step:
Commercial *Eucalyptus* clones are propagated in-vitro and sent to CBD Technologies

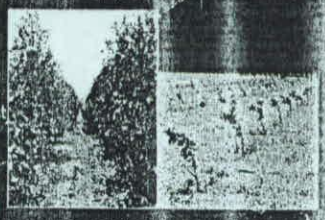
1

Second step:
Development of transformation and regeneration protocol for each one of the clones. Transformation with CBD proprietary genes.

Commercial Eucalyptus Plantation

Seventh step:
Clones with increased biomass and improved fibers from the field test are selected for use in commercial plantations

7



Sixth step:
Field experiments at commercial territories

2

Regenerated transgenic Plant

Third step:
Transgenic plants are isolated and transgene presence is verified.

3

Transgenic plant

Transgenic clone ready for shipping

4

Fourth step:
Transgenic clone are propagated and shipped back to the forestry company

5

Fifth step:
Shipping transgenic clones in sterile tissue culture containers

Transgenic plants are propagated at the Forest company's nursery

6

We have completed protocol development for the following projects:

Partners

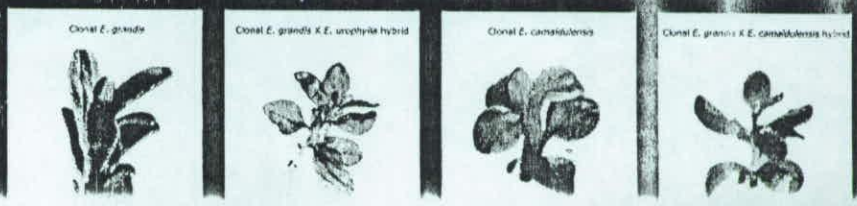
Brazilian partner: *E. grandis* and *E. grandis* X *E. urophylla* hybrid have been transformed with our traits – field test in Brazil is underway.
Thailand partner: *E. camaldulensis* and *E. grandis* X *E. camaldulensis* hybrids have been transformed with our traits – field test in Thailand is planned for next year

Our proprietary germplasm

High yield Brazilian hybrid transformed with our traits - field test in Israel is planned for next year.
* These transgenic trees are available for potential partners.

Independent commercial clones transformed

The plants pictured at the right are transformed with the gus-intron reporter gene.



CBD Technologies

TOWARDS TRANSGENIC EUCALYPTUS TREES IN FOREST PLANTATIONS

Ziv Shani[1], Mara Dekel[1], Noga Barimboim[1], Ofer Cohen[1], Orit Hochberg[1], Oded Shoseyov[2].

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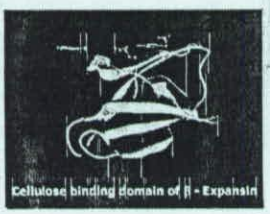
E-mail: zshani@cbd-tech.com

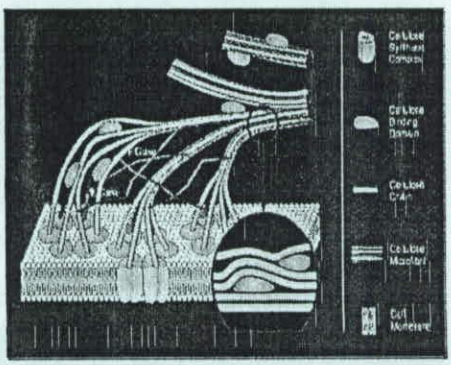
ABSTRACT

CBD Technologies has discovered and developed a novel modality for altering plant traits by modifying plant cell walls. The Company's cell wall modulation platform evolved from research on cellulose binding domains (CBDs), which are families of binding proteins and glucanases that nature designed to facilitate cellulose synthesis and metabolism.
CBD Technologies has utilized these proteins to enhance cellulose production and fiber quality in forest trees and is also developing chemicals based on CBD proteins for use in paper manufacturing and modification.
The Company has entered into several development and commercialization agreements. The first commercial species that the Company is developing is *Eucalyptus*. The Company has already succeeded in transforming its genes into numerous commercial *Eucalyptus* clones of its industrial partners and initiated a field trial in 2003. The Company has formed a strategic partnership with the Brazilian group, Suzano Bahia Sul Papel e Celulose S.A., one of the largest integrated forestry, pulp and paper producers in South America and a development agreement with Tree Tech, a member of the AA Alliance Group in Thailand. By assisting partner forestry companies to significantly improve the yields of their sustainable plantation forests, the CBD technology can help to meet growing fiber demand, without resorting to accelerated destruction of native forests. In addition, rapidly growing forests, utilizing CBD-modified trees are capable of sequestering more CO_2, thus providing additional environmental benefit in ameliorating the global greenhouse effect and potential economic benefit to forestry companies via carbon credits.

TECHNOLOGY AND INTELLECTUAL PROPERTY POSITION

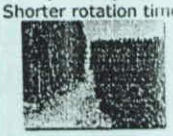
Cell-wall modifying proteins such as bacterial CBDs, expansins, expansin-like proteins and different glucanases are capable of loosening the stiff cell-wall thus accelerating plant cell growth and development.
CBD Technologies Ltd. holds world-wide plant cell-wall modification patents.





OUR AIM:

Improved yields
Shorter rotation time



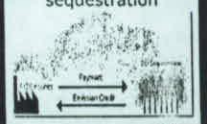
Shorter forest to mill distance

Less growing area

Fundamental cost saving

Preserve natural biodiversity

Enhanced carbon sequestration



Enhanced environmental sustainability & income opportunity

Clonal propagation of mature maritime pine

Major factors contributing to micrografting success

IUFRO Tree Biotechnology Conference, 6-11 November 2005, Pretoria (South Africa)

TRONTIN JF, QUONIOU S, DUMAS E, HARVENGT L
AFOCEL, France. Biotechnology lab (biotech@afocel.fr), Domaine de l'Etançon, 77370 Nangis

OBJECTIVES and METHODS

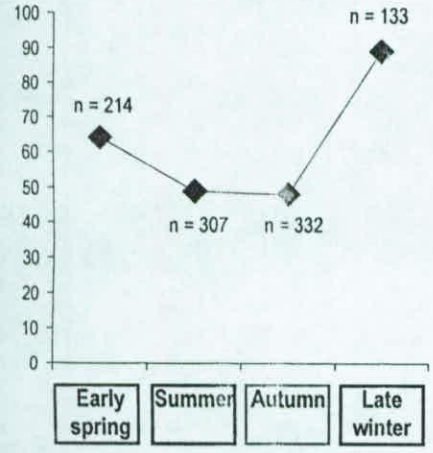
Micrografting of bud meristems onto juvenile rootstocks is currently the best option for efficient *in vitro* establishment of mature trees in maritime pine because most genotypes are responsive. The interest of this technique for both practical application and basic research mainly arises from its potential to rejuvenate and/or reinvigorate and thus to restore the morphogenetic competence of the mature plant material. Unfortunately, micrografting is a technically complex procedure with quite low throughput. Full practical benefits are thus expected only when optimal conditions for micrografting maritime pine are defined.

The objective of this work was to identify some major factors contributing to the final Micrografting Success Rate (MSR) of 1- to 2-year-old grafts from mature, field-tested donor trees.

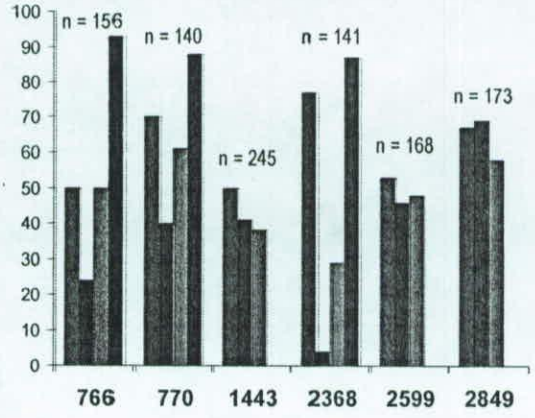
The 6 selected elite genotypes from the breeding program used in this study (766, 770, 2368, 1443, 2599, 2849) were 17 to 30 years old at the time of *in vitro* micrografting. The technique used to obtain micrografts from apical meristems was previously described by Dumas *et al.* (1989, C.R. Acad. Sci. Paris (III) 309: 723-728).

RESULTS (in % MSR = scion development observed 2 months after micrografting; n = sampling size)

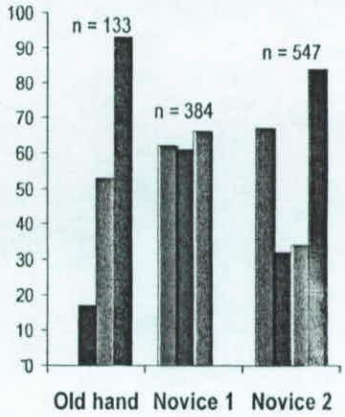
1- SEASON



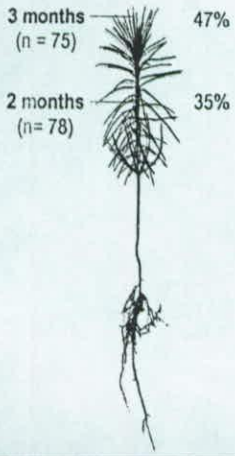
2- GENOTYPE



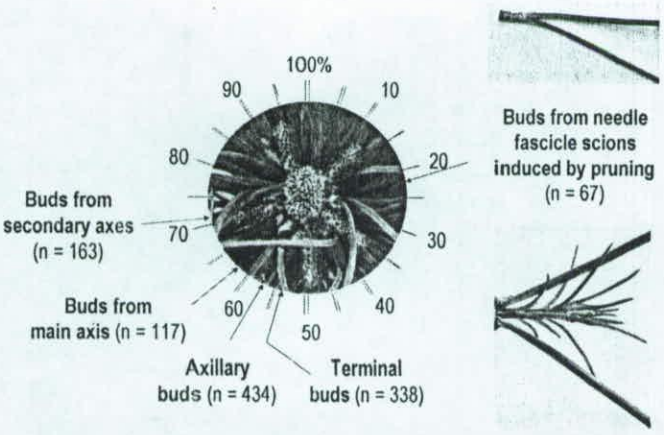
3- OPERATOR



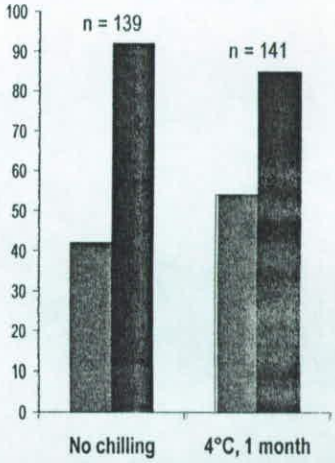
4- ROOTSTOCK AGE



5- BUD TYPE AND POSITION



6- BUD CHILLING



Conclusion

As observed in *Pinus radiata* (Fraga *et al.* 2002, Ann. For. Sci. 59: 155-161), season when buds are collected was apparently the main factor affecting MSR in strong interaction with genotype and operator. Results were greatly improved in late winter (89%) compared to early spring (64%), summer (49%) or autumn (48%). Interestingly, minimal differences were observed during the best sampling seasons (late winter to early spring) among genotypes (≤ 13%) or operators (≤ 10%). Additional variations factors with more limited impact on MSR were revealed such as rootstock chronological age (+12% MSR using 3-month-old vs. 2 month-old seedlings) and bud position on grafted mother plant (+9% using buds collected on secondary axes vs. main axis). No significant difference was observed between terminal (54%) and axillary buds (57%). Buds from needle fascicle scions induced by pruning were found inappropriate (low MSR compared to other buds). Bud storage in chilling condition (4°C) for 1 month prior to micrografting did not improve MSR except in autumn

Accomplishments and challenges of conifer somatic embryogenesis for the implementation of multi-varietal forestry

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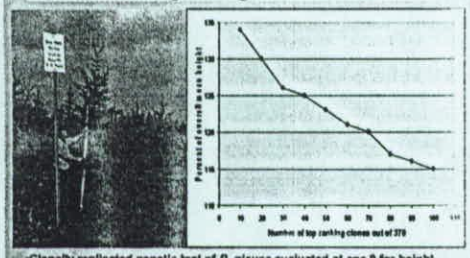
Multi-Varietal Forestry

Multi-varietal forestry (MVF) may be defined as the use of genetically tested tree varieties in commercial plantation forestry.

Advantages of MVF

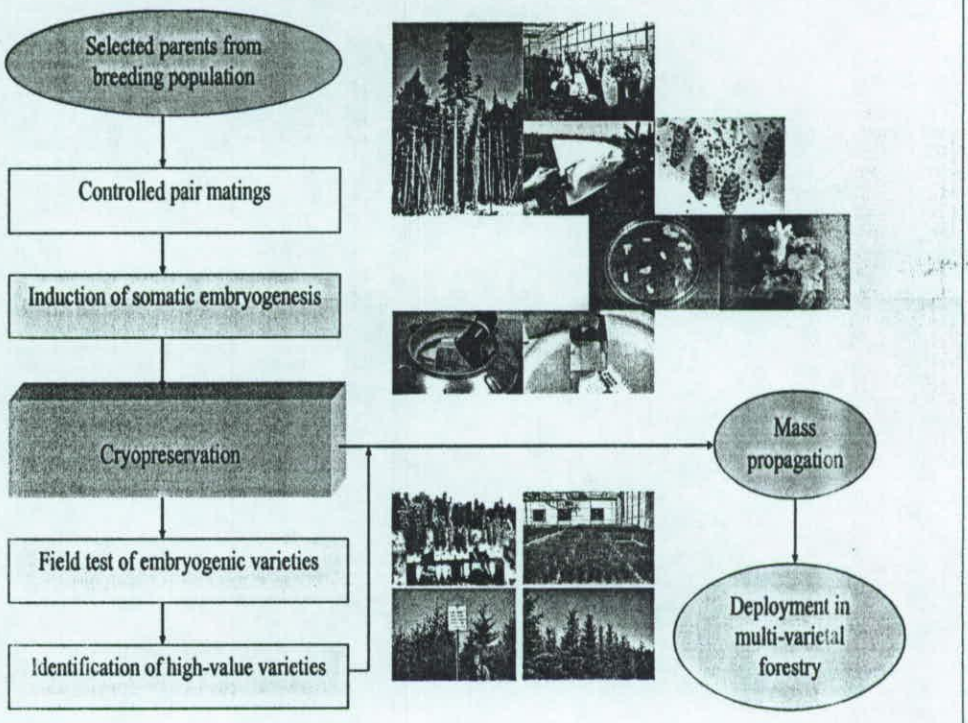
There are many advantages to MVF, including:
- much greater genetic improvement than is possible through conventional tree breeding techniques;
- suitable varieties can be rapidly introduced to meet changing breeding goals, site conditions, and environment change;
- diversity in plantations can be carefully managed by using appropriate mixtures of tested varieties in time and space.

Potential genetic gains with MVF



- Clonally replicated genetic test of *P. glauca* evaluated at age 9 for height
- Test included 370 clones from 72 full-sib crosses
- Test planted at 3 locations with 16 ramets per location

Schematic presentation of implementing MVF using somatic embryogenesis



Challenges and Accomplishments

Somatic embryogenesis and cryopreservation are the primary enabling technologies for implementing MVF. The implementation of MVF requires four critical steps:

Step 1. The development of a sufficiently refined SE system must be achieved, and this is currently available for several conifer species.

Achievements in SE initiation and conversion rates

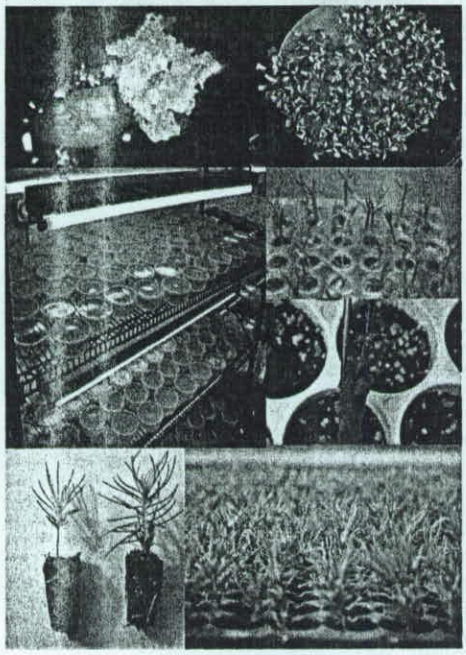
Species	Immature ZE	Mature ZE	Conversion
Picea glauca	68%	20%	87%
P. mariana	65%	21%	87%
P. abies	78%	29%	80%
Pinus strobus	61%	2%	70%
P. resinosa	6%	0%	75%
P. banksiana	4%	0%	80%
P. rigida	36%	0%	80%
P. pinaster	76%	0%	77%
P. sylvestris	20%	0%	80%

Step 2. The development of high-value varietal lines is required, which involves tree breeding and field testing while maintaining SE lines being tested in cryogenic storage.

Varietal testing scheme in NB, Canada

- *200-300 varieties from 20-30 elite crosses*
- *3-4 test sites in New Brunswick*
- *12 ramets of each variety are planted per site*
- *Evaluation every 5 years*
- *Currently, over 2,000 lines in the field tests*

Step 3. Mass vegetative propagation must be achieved in a cost-effective manner. Artificial seed and an automated embryo handling system are currently being developed, e.g., *micro-plug system*.



Step 4. Deployment and management of diversity in MVF. The deployment of embryogenic varieties in plantations requires a careful balancing act to optimize genetic gain yet maintain plantation diversity. Once an appropriate number of varieties has been decided, a deployment strategy must consider the configuration of deployed varieties. Over time, diversity among plantations will also be managed by introducing new clones during each breeding cycle.

Public perception and issues of MVF

"The deployment of SE varieties may lead to increased vulnerability to insects and diseases"
- *For known pests, MVF is better prepared by deploying resistant varieties*
- *For unknown or introduced pests, protection is limited despite the large variability in forest trees; however, the deployment of multi-vaietal mixtures may alleviate the problem*

Deployment strategies for MVF

- *Mosaic of Monoclonal Mixtures (MOMs)*
- *Widespread Intimately Mixed Plantations (WIMPs)*
- *Mixture of Varieties and Seedlings (MOVAS)*
- *Desired Gain Mixtures – Set a level of genetic gain to maintain a desired level of diversity*
- *Linear Deployment – Greater representation of better-known varieties*
- *Species Mixture*

Natural Resources Canada | Ressources naturelles Canada
INRA
Canada

Transformation Systems for the Creation of Somatic Wood Sectors in Trees can Aid the Functional Analysis of Genes Involved in Xylogenesis

THE UNIVERSITY OF MELBOURNE

Kim S. Van Beveren, Antanas V. Spokevicius, Josquin Tibbits and Gerd Bossinger
The University of Melbourne – School of Forest and Ecosystem Science, Water St, Creswick, Victoria, Australia 3363

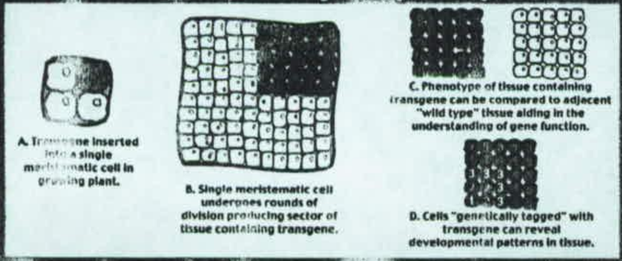
1. Introduction

Functional analysis of genes involved in xylogenesis in tree species using current techniques is hindered by long re-generation times, high technical and labor demands and difficulties in propagating woody plants.

The use of Induced Somatic Sector Analysis (ISSA) can circumvent some of the problems associated with plant regeneration and maturation by directly transforming cambial tissue in the stem.

Using *Agrobacterium tumerfaciens*-mediated transformation protocols we describe *in vitro* and *in vivo* methods for the elucidation of gene function in woody tissue of commercially important trees in a matter of months.

2. The concept of Induced Somatic Sector Analysis (ISSA)

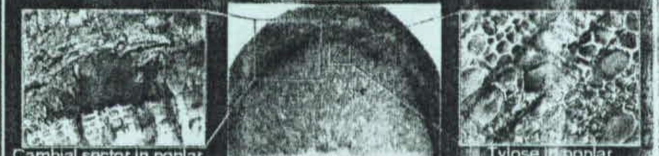


3. *In vitro* Apical Stem Segment system

This system utilises *in vitro* Apical Stem Segment techniques where cambial activity can be induced in culture[1]. After samples have been prepared for culturing they are exposed to *A. tumerfaciens* followed by clean up protocols[2]. Explants undergo wound closure and resume normal cambial activity producing transgenic xylem and phloem derivatives. Techniques have been successfully applied in both angiosperm and gymnosperm species.

1. Stem harvested and sterilised. 2. Stems cut and exposed to *A. tumerfaciens*. 3. Explants cleaned and cambial growth resumes.

GUS visualisation of transgenic tissue 12-20 days after exposure to *A. tumerfaciens*



Cambial sector in poplar — Tylose in poplar

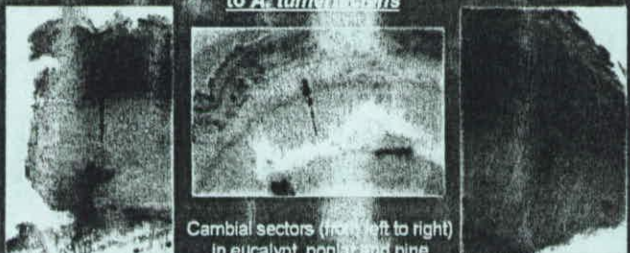
1. Leitch MA, Bossinger G (2004). In vitro systems for the study of wood formation. In: [illegible]
2. Spokevicius AV, Van Beveren K, Leitch MA, Bossinger G (2005). [illegible]

4. *In vivo* stem system

In this system cambial cells in the stem of growing plants are exposed to *A. tumerfaciens* via wounding techniques. After wound closure the cambium resumes activity producing transgenic xylem and phloem derivatives. Techniques have been successfully applied in both angiosperm and gymnosperm species.

1. Cambium exposed via bark pealing. 2. *A. tumerfaciens* introduced into wound site. 3. Bark strip placed back into slot and wrapped to aid recovery.

GUS visualisation of transgenic tissue 120-240 days after exposure to *A. tumerfaciens*

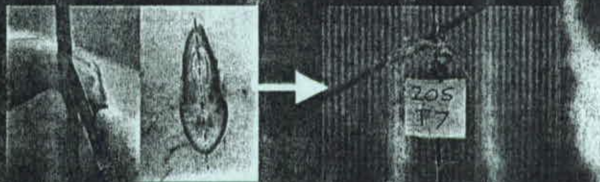


Cambial sectors (from left to right) in eucalypt, poplar and pine

Phloem sectors in eucalypt (left) and poplar (right) — Wound parenchyma sector in eucalypt — Tyloses in poplar

5. *In vivo* dormant lateral bud system

This system is similar to the *in vivo* stem system except in this case meristematic tissue within a dormant lateral bud is targeted for *A. tumerfaciens* inoculation via cutting techniques. This systems targets cells earlier in development with the potential of producing larger sectors of transgenic tissue. Techniques are limited to deciduous angiosperms species with large, robust dormant lateral buds.



1. Buds cut longitudinally (along dashed line) and exposed to *A. tumerfaciens*. 2. Buds are covered in protective wrapping and allowed to develop.

GUS visualisation of transgenic tissue 51-125 days after exposure to *A. tumerfaciens*

Cambial sector in poplar

Outer cortex sector in poplar — Pith sector in poplar

Leaf sector in poplar

For further information [illegible]
Spokevicius AV, Van Beveren KS, Bossinger G (2006). [illegible]

6. Conclusion

We show that Induced Somatic Sector Analysis (ISSA) can be used successfully to introduce genes into secondary tissue with the production of transgenic wood sectors for functional analysis

Contact Us

Feel free to contact us with any questions or queries about the methods presented here. We look forward to hearing from you.

Kim Van Beveren: ksvb@unimelb.edu.au

GOBIERNO DE CHILE
FUNDACIÓN PARA LA INNOVACIÓN AGRARIA

IUFRO Tree Biotechnology

Pretoria, Sudáfrica
6 al 11 de noviembre, 2005

Organización

- IUFRO Divison 2, Unit 2.04.06, Molecular biology of trees.
- Universidad de Pretoria

Alexander Myburg

Asistentes - FIA

Facultad Ciencias Forestales - Centro de Biotecnología - Universidad de Concepción:

- Jaime Rodríguez
- Regis Mendonca
- Juan Pedro Elissetche
- Sofía Valenzuela

Pontificia Universidad Católica de Chile:

- Felipe Aquea

Simposios

- Tree biotechnology in the postgenomic era
- Tree interactions with pests, pathogens and symbionts
- Breakthrough and high-throughput technologies for functional and structural genomics in trees

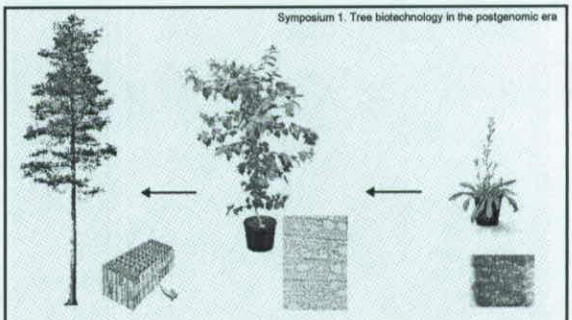
Simposios (cont.)

- Forest biotechnology adoption and the impact of the economic, scientific and societal value chains
- Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

Simposios (cont.)

- Molecular biology and biotechnology of tree development
- From somatic embryos to genetic engineering
- Biotechnology and metabolic engineering of wood formation in trees
- Abiotic stress: Interaction of trees with the environment

Symposium 1. Tree biotechnology in the postgenomic era



Coníferas	Álamo	*Arabidopsis thaliana*
15.500 Mpb	550 Mpb	125 Mpb

Entidades participantes:

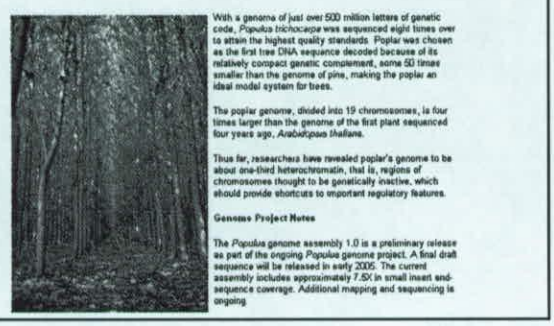
DOE joint genome institute

DOE Oak Ridge national laboratory

Genome Canada

Umea Plant Science Centre

Ghent University



SI.1

FROM GENOME SEQUENCE TO TRANSCRIPTOME: STUDIES USING A WHOLE-GENOME POPLAR MICROARRAY

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3 - Virginia Tech Department of Forestry 310A Cheatham Hall (0324) Blacksburg, VA 24061

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-Desarrollo de matriz que contiene todo el genoma de Populus y ESTs de Aspen.

-La matriz contiene 65.966 secuencias individuales (genes nucleares, organelares y precursores de MicroRNA)

-Hibridación heteróloga con otras especies de Populus.

-Primeros experimentos identificaron 4000 genes expresados diferencialmente entre xilema y floema.

S1.21p

SEQUENCING OF THE *EUCALYPTUS* TRANSCRIPTOME IN THE GENOLYPTUS PROJECT

Giancarlo Pasquali[1], Fernanda M. Bastolla[1], Rochele P. Kirch[1], Rosana V. Brondani[3] Alexandre S.G. Coelho[3], Dario Grattapaglia[4], Sérgio H. Brommonschenkel[5], Georgios J Pappas Jr.[6], Gonçalo A.G. Pereira[7], Júlio C. M. Cascardo[8]

1 - Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre. RS Brazil
2 - EMBRAPA Arroz e Feijão, Goiânia, GO, Brazil
3 - Dept. Biologia Geral, Universidade Federal de Goiás, Goiânia, GO, Brazil
4 - EMBRAPA Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil
5 - BIOAGRO, Universidade Federal de Viçosa, Viçosa, MG, Brazil
6 - Lab. Bioinformática, Universidade Católica de Brasília, Brasília, DF, Brazil
7 - Lab. Genética e Expressão Gênica, UNICAMP, Campinas, SP, Brazil
8 - Lab. Genômica e Expressão Gênica, UESC, Ilhéus, BA, Brazil

S1.8

SAMPLE SEQUENCING OF 3 MEGABASES OF SHOTGUN DNA OF *EUCALYPTUS GRANDIS*: GENOME STRUCTURE, REPETITIVE ELEMENTS AND GENES

Rodrigo T. Lourenço[1,2], Dario Grattapaglia[2,3], Georgios J. Pappas Jr[3], Gonçalo A. Pereira[1]

1 - UNICAMP-Universidade de Campinas, Dep. Genetics and Evol., 13083-970 Campinas, SP, Brazil
2 - Embrapa Genetic Resources and Biotechnology, W5 Norte 70770-970 Brasília, DF, Brazil
3 - UCB-Universidade Católica de Brasília, SGAN 916, 70790-160 Brasília, DF, Brazil

E-mail: gpappas@bioinformatica.ucb.br

S1.3

IDENTIFICATION OF GENES CONTROLLING WOOD FIBRE PROPERTIES IN *EUCALYPTUS NITENS*

Deyou Qiu[1], Iain Wilson[2], Russell Washusen[2], Gavin F. Moran[3], Simon Southerton

Ensis – A joint venture between CSIRO and SCION, PO Box E4008 Kingston, ACT 2604, Australia

1 - Current Address: The Research Institute of Forestry, Chinese Academy of Forestry, Beijing, 100091, P.R.China
2 - CSIRO Plant Industry, Black Mountain, ACT 2601, Australia
3 - Ensis, Bayview Ave, Clayton VIC, Australia
4 - School of Botany and Zoology, The Australian National University, Canberra ACT 0200, Australia

E-mail: simon.southerton@csiro.au

-Chip microarrays contiene 4500 cDNAs
-Análisis de expresión entre ramas y tronco.
-Genes identificados:
1.-Tubulina
2.-Proteoglicanes
- Posible función: influenciar la orientación de celulosa

Symposium 3. Breakthrough and high-throughput technologies for functional and structural genomics in trees

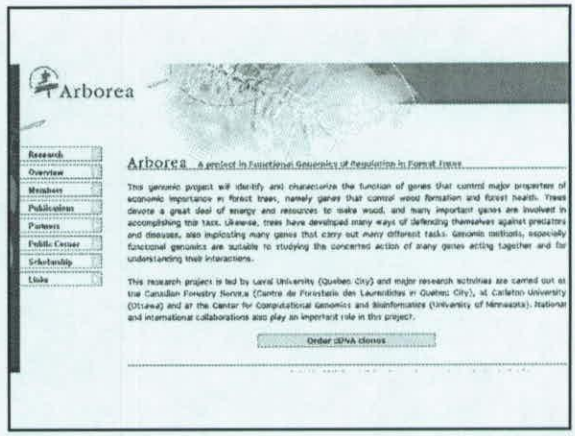
S3.2

A FUNCTIONAL GENOMICS APPROACH TO IDENTIFYING POTENTIAL REGULATORS OF TREE GROWTH, DEVELOPMENT AND DEFENSE RESPONSES

John MacKay[1], Caroline Levasseur[2], Laurence Tremblay[2], Janice Cooke[3], Armand Séguin[2]

1 - Centre de Recherche en Biologie Forestière, Université Laval, Sainte-Foy QC G1K 7P4 Canada
2 - Natural Resources Canada, Laurentian Forestry Centre, 1055 rue du P.E.P.S., Sainte-Foy QC G1V 4C7 Canada
3 - University of Alberta, Department of Biological Sciences, Edmonton AB T6G 2E9, Canada

E-mail: seguin@cfl.forestry.ca



Arborea

OBJETIVOS PRINCIPALES:

1.- Identificación de genes

2.-Estudiar la función de genes identificados en:

- Formación de madera
- Respuesta de defensa a patógenos

ESPECIES:

1. Populus
2. White spruce

Arborea

-**Formación de madera:**

Estudio de reguladores candidatos para la diferenciación de xilema.

Factores de transcripción: HD zip – MybR2R3 – Knox 1 – Scarecrow

Sobreexpresión de genes en Spruce:

Myb 8: Aumento en la lignificación de raíces

Dominio LIM: Alteración en el grosor de la pared celular.

Arborea

-Respuesta de defensa:

Estudio de reguladores candidatos para la respuesta de defensa.

Factores de transcripción: WRKY – TGA – Mybs- MAPKK

MELAPSORA

RUST

S3.4

FUNCTIONAL GENE TESTING IN *PINUS RADIATA* CALLUS CULTURES

Armin Wagner, Ralf Möller, Diane Steward

Cellwall Biotechnology Centre, Scion, Te Papa Tipu Innovation Park, Private Bag 3020, Rotorua, New Zealand

S3.4

FUNCTIONAL GENE TESTING IN *PINUS RADIATA* CALLUS CULTURES

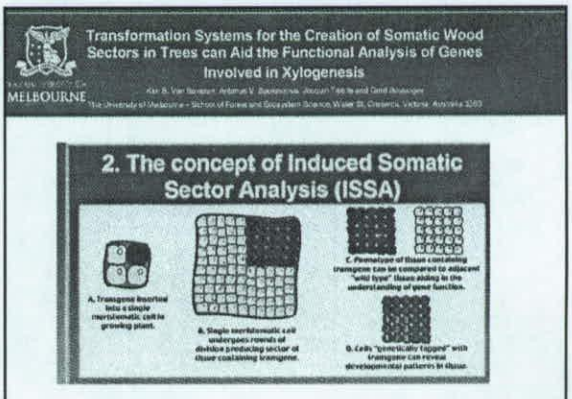
Estudio funcional de genes relacionados con pared celular (mediante silenciamiento génico):

Pasos:

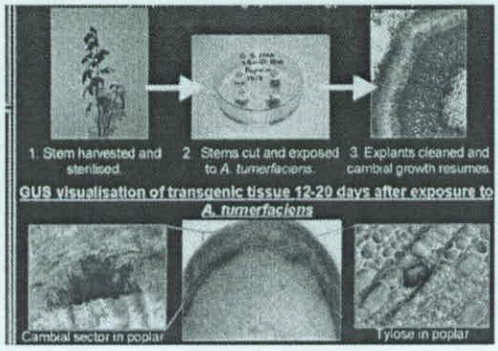
Transformación ⟶ Diferenciación ⟶ Purificación ⟶ Análisis

Solo se requiere la expresión transiente del transgén

Desventaja: Información del fenotipo limitada



Análisis de segmentos apicales *in vitro*



4. *In vivo* stem system

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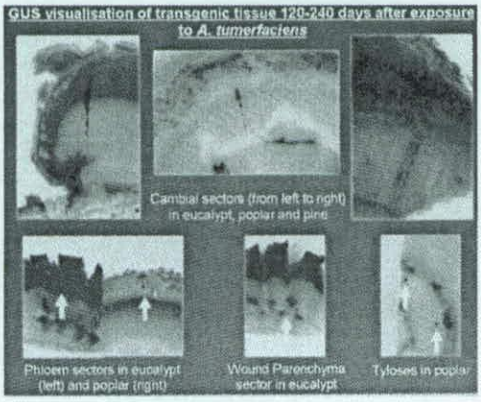

Symposium 4. Forest biotechnology adoption and the impact of the economic, scientific and societal value chains

S4.2

THE BENEFIT OF THE APPLICATIONS OF BIOTECHNOLOGY TO FORESTRY

Maud Hinchee

Chief Technology Officer, ArborGen LLC., PO Box 840001, Summerville, SC, USA

ArborGen ... The Future of Forestry

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ArborGen's vision is to create products that benefit customers, sustain the environment, and provide value to the forest industry through the application of genetics and enhanced trait technology. The application of research provides an additional tool in the sustainable forestry toolbox.

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Interesados en " fast-wood forestry":

-Innovación en prácticas silvoagrícolas

-Mejoramiento genético asistido por marcadores

-Transgénicos.

-Embriogénesis somática

Objetivo:

-Reducir contenido de lignina

-Reducir tiempo de rotación (en Pinus taeda de 26 a 6 ó 9 años)

-Modificar pared celular (aumento en el crecimiento)

-Inducir esterilidad (evitar propagación de transgénicos)

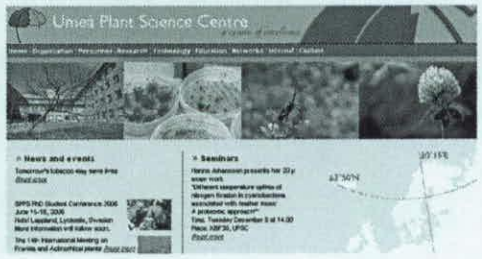
Symposium 6. Molecular biology and biotechnology of tree development

S6.2

ANALYSIS OF THE REGULATION OF CAMBIAL MERISTEM ACTIVITY USING A GENOMICS APPROACH

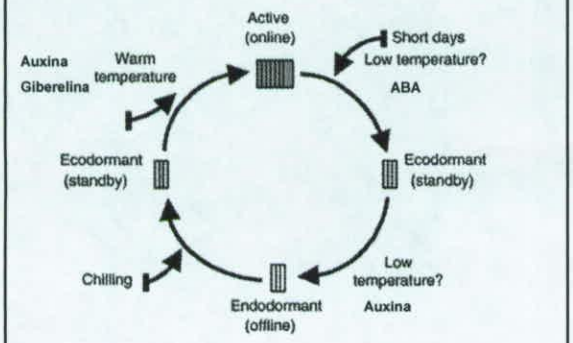
Rishikesh P. Bhalerao, Jarmo Schrader, Lars Resman, Nathalie Druart, Annika Johansson, Thomas Moritz, Kyoko Baba, Glenn Howe, Tony Chen, Goran Sandberg

Umea Plant Science Center, S-901 83, Umea, Sweden and OSU, Corwallis, USA



S6.2

ANALYSIS OF THE REGULATION OF CAMBIAL MERISTEM ACTIVITY USING A GENOMICS APPROACH

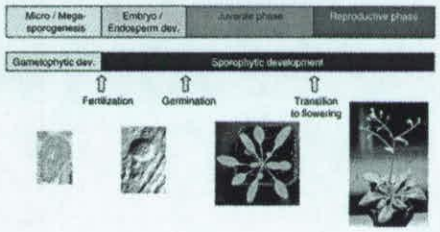


S6.5

REGULATION OF FLOWERING TIME IN TREES

Henrik Böhlenius, Tao Huang, Laurence Charbonnel-Campaa, Ove Nilsson

Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90183 Umeå, Sweden



S6.5

REGULATION OF FLOWERING TIME IN TREES

ASPEN

-Sobreexpresión del gen *LEAFY* induce floración temprana (9 meses)

-Sobreexpresión del gen *FT* induce floración extremadamente temprana (4 semanas)

35S:: FT produce catkins a 6 meses

S2. Tree interactions with pests, pathogens and symbionts

Temas

Interacción planta-huésped
(Señales de respuesta)
Elizabeth Magel (Alemania)

Hongos patógenos
(secuenciación del genoma y genes de interés)
Jan Stenlind (Suecia)
Carl Gunnar Fossdal (Noruega)

Respuesta de la planta frente a patógenos
(proteómica)
Armand Seguin (Canadá)

Principales casos de estudio

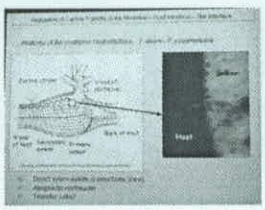
Hongos patógenos

Heterobasidion annosum
Puccinia psidii
Phytophtora spp.
Dothristroma spp.
Fusarium sp.

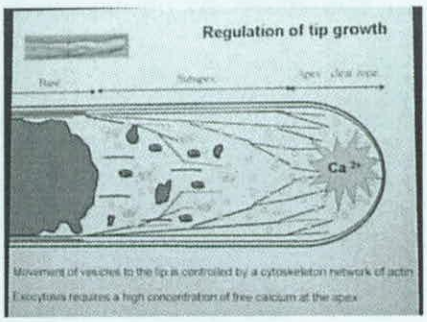
Involucrados algunos en pitch canker con pérdidas económicas
En plantaciones forestales de coniferas.

Interacción planta-hueped

Viscum album



Señales de respuesta frente al parasitismo y su efecto sobre el hospedero
•Genes asociados a modificaciones químicas y morfológicas del hospedero

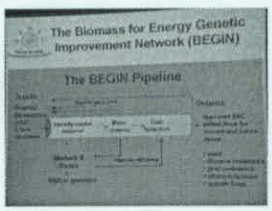


Factores genéticos y ambientales que participan en la regulación del crecimiento de las hifas durante el ataque de patógenos (V. Legué, Francia)

S5. Genome-directed tree improvement: Application of genomics to understand the genetics, evolution and ecology of tree populations

Análisis genéticos en "willow" para bioenergía

- Proyecto del UK Biomass for Energy Crop Improvement Network (BEGIN)

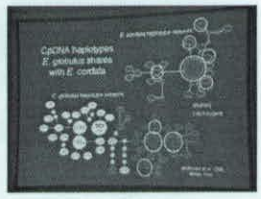


S. Hanley, Reino Unido

- Empleo de tecnologías moleculares para mejorar características en el corto plazo.
- Progenie de *S. viminalis x S. schwerinii*

Aspectos moleculares en el pool genético de *E. globulus*

- Marcadores de ADNcp para estudiar la historia evolutiva de *E. globulus*.
- Posibles hibridaciones con otras especies a lo largo de la evolución.



Potts et al, Australia

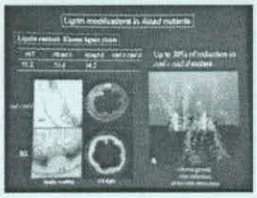
S8. Biotechnology and metabolic engineering of wood formation in woods

Principales áreas

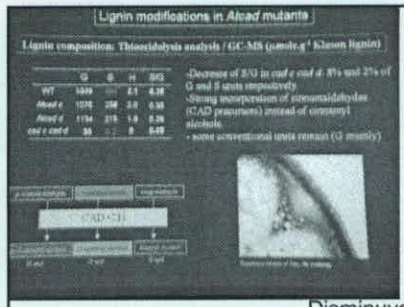
- Vías genéticas de síntesis de lignina
- Transformación de plantas modelos
- Estudios pared secundaria

Arabidopsis CAD mutant

- 9 genes de CAD en Arabidopsis
- Mutante en dos de los genes At cad cd, disminuye significativamente niveles y composición de lignina.



Lise Jouanin, INRA, Francia



Disminuye S/G en mutantes cad c y cad d en un 8% y 2%, respectivamente.

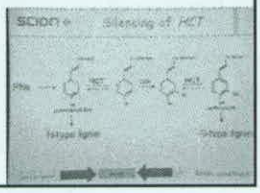
Down regulation of Cinnamoyl-CoA-reductase in poplar: from the lab to the field.

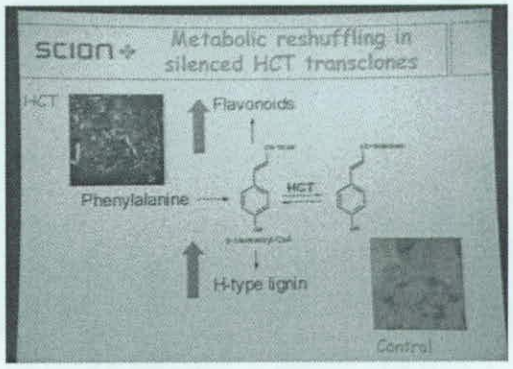
- CCR, reduce lignina, afecta la estructura celular y crecimiento.
- Ensayos de campo hay 4 líneas (4 años)

Reducción de lignina por diferentes vías

CCR (Boerjan et al., Bélgica)

HCT (Scion, Nueva Zelanda)





Conclusions I

-> CCR down-regulation results in 20-40% less lignin

-> the reduced flux translates into accumulation of storage products and the disappearance of oligolignols

-> Thioacidolysis and immunolocalization of lignin epitopes indicate lignin is more condensed

-> the cell wall ultrastructure is affected

Q: Is the defect in cell wall structure merely due to the physical absence of lignin?

Conclusions II

-> Defects in a structural gene of secondary metabolism affect levels of many transcripts and metabolites, revealing interactions among pathways.

-> These alterations may explain part of the phenotype, eg cell wall ultrastructure may be due to:

1. reduced lignin levels per se -> less cohesion in CW
2. reduced lignin levels signal (e.g. via AGP) to the nucleus to reduce hemicellulose levels

-> how do these plant behave in the field (pulping and growth

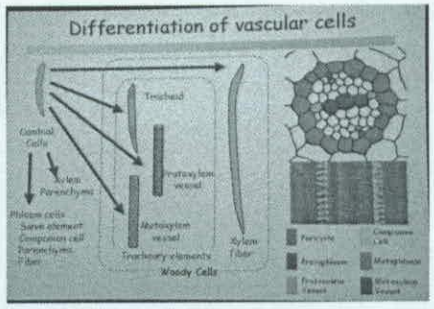
Conclusions III

-> Down-regulation of CCR reduces lignin content

-> This translates to improved pulping (lower Kappa, higher pulp yield, less uncooked particles)

-> But, ...growth is affected: knock-outs are too strong

-> molecular breeder needs to exploit allelic diversity



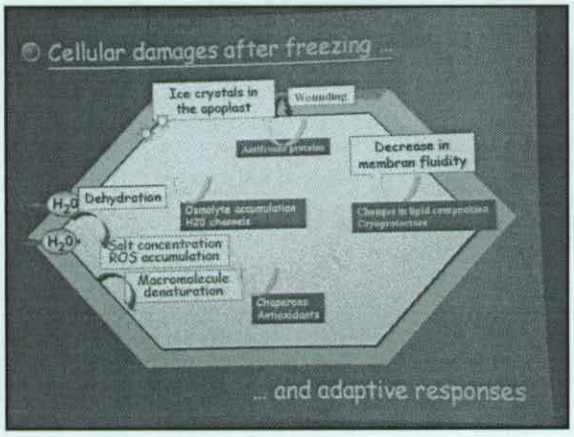
Genes involucrados en la diferenciación celular

S9. Abiotic stress: Interaction of trees with the environment

CBF transcription factors on *Eucalyptus* cold tolerance.

- Comprender el mecanismo involucrado en la tolerancia al frío e identificar marcadores moleculares (SNPs) para MAS.
- Resultados en otras especies indican asociación de genes involucrados en diferentes tipos de estrés abióticos.

Marque et al., University of Toulouse, Francia



366.000 ESTs (3 librerías de cDNA), identificaron ca. 2.465 ESTs asociados con respuesta al frío.

- CBF, 2 identificados inducidos por frío, y en menor grado por salinidad o ABA.
- Patrón de expresión difierente según sea el nivel de inducción de frío.
- Búsqueda de SNPs para tolerancia al frío.

Using functional genomics to dissect drought responses in pine

- 172.000 ESTs de raíz de pino taeda, sometido a diferentes estreses físicos (sequía, frío, oscuridad), químicos (exceso de metales pesados) y nutricional.
- Identificaron 25.000 secuencias únicas
- Microarreglos con 13.500 genes

– J. Dean et al. Georgia, USA

Control genético en plasticidad molecular en pino marítimo

- Empleando geles 2D de alta resolución, un total de 1039 spots fueron detectados.
- De éstos (analizados por MS/MS) :
  - 67.9% fueron identificados
  - 16.7% sin homología en bases de datos
  - 15.4% mezclas de proteínas.

La mayoría de las proteínas identificadas (175) juega un rol en defensa (19.4%), metabolismo de carbohidratos (16.6%) y amino ácidos (14.9%), citoesqueleto (8%), biosíntesis pared celular (5.7%).

Populus euphratica compensa el estrés inducido por salinidad

- *P. euphratica* tiene una alta tolerancia a salinidad, pero baja tasa de crecimiento.
- Estudio de antiporter Na+/H+
- Acumulación de sodio en la pared celular, resultando en menor captura de Ca

Reunión
"Consorcio Internacional Genómica de Eucaliptos"

IEuGC

Aspectos relevantes

- D.O.E. de los E.E.U.U., dispuesto a secuenciar genoma de plantas, entre ellos una especie de eucalipto
- Biblioteca EST pública?. La posición de Genolyptus
- Genoma de *E. calmadunensis* (Japón)

Acuerdos

Enviar propuesta al DOE, durante diciembre 2005.

Definir especie a estudiar de eucalipto

Empresas – Universidades liderando el area

- Genolyptus, Brasil
- Arborgen, USA
- Scion, Nueva Zelanda
- SweTree, Suecia
- Umea Plant Science Center, Suecia.
- Natural Resource Canadá
- Oak Ridge National Laboratory, USA
- Ensis (Joint venture CSIRO & SCION, Australia)

Conclusiones

- Estudios genéticos en diferentes especies, énfasis en álamo, pino y eucalipto.
- Desarrollo de bibliotecas génicas, secuenciación de algunas especies.
- Empleo de herramientas moleculares (microarreglos, ESTs, marcadores) para comprender la genómica funcional.
- Estudios en :
  - Aumentar producción de celulosa
  - Resistencia a estreses bióticos y abióticos
  - Supresión de genes en vía de biosíntesis de la lignina

Próximo Congreso

- IUFRO Tree Biotechnology 2007
  - Azores, Portugal

Agradecimientos

- FIA, Programa de Captura y Difusión Tecnológica
- Programa MECESUP (UCO/UACH)
- Escuela de Graduados, Universidad de Concepción
- Laboratorio de Genómica y Biología Molecular.