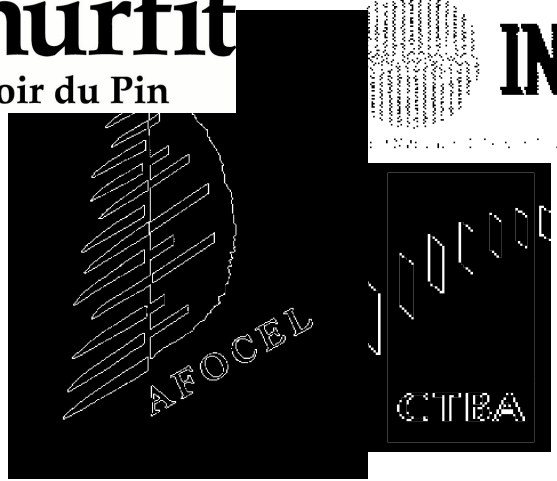
Wood, Breeding, Biotechnology and industrial expectations

A Joint Meeting of:

- EU funded projects
- The 9th Conifer Biotechnology Working Group
- IUFRO Working Parties
  - 2.04.00: Genetics
  - 5.01.02 : Natural variations in wood quality

La Cité Mondiale
Bordeaux, France
June 11-14, 2001

Smurfit
Comptoir du Pin



INRA

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OFFICIAL PROGRAM
WBB conference

Organiser: Christophe Plomion (INRA)

Sponsored by:

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ORAL PRESENTATIONS

DAY 0 : (10/06/01)	Arrival	
17.00-19.00	Registration, Poster mounting, Loading powerpoint presentations	
19.00-22.00	Welcome mixer	
DAY 1: (11/06/01)	**Plenary session, S1**	
8.00-18.30	Registration, poster mounting	
9.00-9.30	Opening ceremony **C Plomion** (F) and **A Franc** (F)	
9.30-10.00	keynote #1 : Tree genetics in the Omics Era. **T Richardson** (NZ)	
10.00-10.40	keynote #2 : Somatic embryogenesis for large-scale clonal testing and propagation of elite material. **J Aitken** (NZ)	
10.40-11.00 *Coffee/Tea break*		
11.00-11.40	keynote #3 : Genetics of eucalyptus wood properties. **C Raymond** (AU)	
11.40-12.20	Keynote #4: Weather, Water, Wood: Environmental effects on wood and fibre properties. **R Wimmer** (AT)	
12 :20-14 :00 *Lunch*		
14.00-14.40	Keynote #5 : Industrial Perspectives and Application of Biotechnology for Sustainable Production of High Quality Wood. **D Carraway** (US)	
14.40-15.20	Keynote #6: Arabidopsis and candidate genes for Wood Quality QTLs in maritime pine. **H Höfte** (F)	
15.20-16.00	keynote #7 : EST-sequencing, micro-array analysis and wood formation in Populus. **B Sundberg** (S)	
16.00-18.30	**Poster session** : presenters with ODD numbered posters should be at their posters (*Coffee/Tea/Refreshment* provided)	
18.30-20.30	Conifer Biotech Working Group business meeting (M Pâques, MA Lelu)	
20.30	free	
DAY 2: (12/06/01)	**Parallel Sessions** **S2/S3/S4/S5**	**S6/S7/S8**
8.00-8.20	J Launay (F) - S2 -	R Timmis (US) – S6 -
8.20-8.40	C Matheson (AU) - S2 -	S von Arnold (S) – S6 -
8.40-9.00	F Bos (F) - S2 -	H Ross (D) – S6 -
9.00-9.20	A Koubaa (CA) - S2 -	J Find (DK) – S6 -
9.20-9.40	SO Lundqvist (S) - S2 -	K Szczygiel (PL) – S6 -
9.40-10.10 *Coffee/Tea break*		
10.10-10.30	P Castera (F) - S2 -	V Chalupa (CZ) – S6 -
10.30-10.50	X Huang (UK) - S2 -	M Gaurav (IN) – S6 -
10.50-11.10	R Evans (AU) – S2 -	C Ramirez-Serrano (MX) – S6 -
11.10-11.30	M Tiitta (FIN) - S2 -	L Harvengt (F) – S6 -
11.30-11.50	T Fourcaud (F) - S2 -	G Pullman (US) – S6 -
11.50-12.10	H Baillères (F) - S2 -	K Klimaszewska (CA) – S6 -
12.10-14.00 *Lunch*		
14.00-14.20	JC Rodrigues (P) - S2 -	O Mashkina (RU) – S6 -
14.20-14.40	H Pereira (P) - S2 -	K Zoglauer (D) – S6 -
14.40-15.00	H Wu (AU) - S3 -	J Nowakoska (PL) – S6 -
15.00-15.20	K Takata (JP) - S3 -	MA Lelu (F) – S6 -
15.20-15.40	Z Kaya (TR) - S3 -	K Zoglauer (D) – S7 -
15.40-16.10 *Coffee/Tea break*		
16.10-16.30	R Volosyanchuk (UA) - S3 -	Y Huang (US) – S7 -
16.30-16.50	L Wilhelmsson (S) - S3 -	C Grünwald (D) – S7 -
16.50-17.10	B Karlsson (S) - S3 -	S Kumar (D) – S7 -
17.10-17.30	B Hannrup (S) - S3 -	S Kontunen-Soppela (FIN) – S7 -
17.30-17.50	P Rozenberg (F) - S3 -	J Grima-Pettenati (F) – S7 -
17.50-18.10	G Chantre (F) - S3 -	C Avila (E) – S7 -
18.30 : Departure by bus to *Château Giscours* (meeting at the main entrance of the conference centre)		
DAY 3 : (13/06/01)	**Parallel Sessions (continuation)**	
8.00-8.20	P Saranpää (FIN) - S3 -	JF Trontin (F) – S7 -
8.20-8.40	F Zamudio (CL) - S3 -	T Aronen (FIN) – S7 -
8.40-9.00	D Cown (AU) – S4 -	Y Helariutta (FIN) – S7 -

9.00-9.20	J Beaulieu (CA) – S4 -	C Dubos (F) – S7 -
9.20-9.40	C Sorensson (NZ) – S4 -	S Nigro (ZA) – S7 -
9.40-10.10 *Coffee/Tea break*		
10.10-10.30	J King (CA) – S4 -	A Altman (IL) – S7 -
10.30-10.50	M Ivkovich (CA) – S4 -	G Le Provost (F) – S7 -
10.50-11.10	M Shepherd (AU) – S5 -	R Herrera (CL) – S7 -
11.10-11.30	E Barros (ZA) – S5 -	JC Leplé (F) – S7 -
11.30-11.50	E Ritter (E) – S5 -	M Fraga (E) – S7 -
11.50-12.10	D Chagné (F) – S5 -	M Campbell (UK) – S7 -
12.10-14.00 *Lunch*		
14.00-14.20	M Sewell (US) – S5 -	M Bou Dagher-Kharrat (F) – S8 -
14.20-14.40	D Pot (F) – S5 -	R Schubert (D) – S8 -
14.40-15.00	F Aravanopoulos (EL) – S5 -	Z Kaya (TR) – S8 -
15.00-15.20	D Prat (F) – S5 -	B Fady (F) – S8 -
15.20-15.40	T Markussen (D) – S5 -	G Vendramin (I) – S8 -
15.40-16.00	R Yazdani (S) – S5 -	S Mariette (F) – S8 -
16.40-16.20	F Villani (I) – S5 -	
16.20-18.00	**Poster session** : presenters with EVEN numbered posters should be at their posters (*Coffee/Tea/Refreshment* provided)	
18.00-20.00	Dinner (free)	
20.00-22.00	**GMO workshop:** acceptation of forest tree GMO in the European Union (Chaired by L Harvengt and M Campbell).	
DAY 4: (14/06/01)	**Plenary session, S9**	
8.00-8.20	MF Deguilloux (F) – S9 -	
8.20-8.40	I Scotti (I) – S9 -	
8.40-9.00	YS Park (CA) – S9 -	
9.00-9.20	J Cairney (US) – S9 -	
9.20-9.40	G Brown (US) – S9 -	
9.40-10.00	J MacKay (US) – S9 -	
10.00-10.30 *Coffee/Tea break*		
10.30-10.50	G Moran (AU) – S9 -	
10.50-11.10	D Verhaegen (F) – S9 -	
11.10-11.30	C Echt (NZ) – S9 -	
11.30-11.50	D Neale (US) – S9 -	
11.50-12.10	W Boerjan (B) – S9 -	
12 :15-14 :00 *Lunch*		
14.00-14.40	Keynote #8: Incorporating Wood Quality Traits into Tree Breeding Programs: Challenges and Opportunities. **T Zhang** (CA)	
14.40-15.20	Keynote #9: Commercial delivery of genetic improvement to conifer plantations using somatic embryogenesis. **B Sutton** (CA)	
15.20-16.00	Keynote #10 : Future developments in forest tree improvements. **R Sederoff** (US)	
16.00	**C Cahalan** (UK), final word	
16.00	*Coffee/Tea break*	

SESSION 1

Chairs, Nino Borralho/ Jenny Aitken

Application of new technology to meet the requirements of the wood chain industry

Corresponding author : Tom Richardson

Dr. Tom Richardson*

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Title: TREE GENETICS IN THE OMICS ERA

Abstract: The major commercially important forest tree species have been the object of genetic improvement programmes for some decades now, and most of these programmes have demonstrated impressive genetic gains for composite traits like growth rate, general form and tree health. However, as many speaker topics and posters at this meeting demonstrate, a ubiquitous theme in current tree breeding programmes worldwide is the heightened emphasis on understanding and improving component wood and fibre traits to enhance processing, performance and predictability of current products, and to develop new wood and fibre properties for next generation products. This shift towards targeting component traits that are naturally later expressing, coupled with the desire to continually shorten the time to market with new traits and products creates some special challenges! Fortunately, a range of new technologies are being developed and applied to meet these needs. Chief amongst the new tools are methods that enable non-destructive, early measurement of wood and fibre characteristics and a host of genetic technologies, largely emanating from human genome projects, that can be used to elucidate the genetic control of component wood and fibre characteristics. In this keynote talk I will use examples from recent work by colleagues at NZ ***Forest Research*** to introduce some of these new genetic tools and to describe how forest scientists are adapting techniques such as gene mapping, transcriptome profiling, comparative genomics and genetic engineering to reveal the genetic control of wood and fibre properties and to select superior individuals at very early ages. Later speakers and posters will detail the application of these tools to a range of species and target traits.

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Aitken-Christie , Jenny[1*]

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* indicates the presenting author(s)

Title : SOMATIC EMBRYOGENESIS FOR LARGE-SCALE CLONAL TESTING AND PROPAGATION OF ELITE MATERIAL

Abstract : Organogenesis of radiata pine has limitations in multiplication rate, genetic transformability and in aging of clonal material. This will likely lead to higher costs, unrealised genetic potential, and loss of growth rates and clones. The full commercial potential of current technology will not be realised, unless substantial research advancement is made to overcome these issues. Global research progress on somatic embryogenesis of conifers has advanced tremendously in the past 10 years by many major forestry companies, universities and research organisations. Thus, the application of new embryogenesis technology will advance at a faster rate than organogenesis-based protocols. The status of organogenesis and somatic embryogenesis tissue culture techniques for radiata pine will be compared. One of the greatest attributes of the somatic embryogenesis technology is the ability to successfully cryostore juvenile embryogenic tissue, which has a low physiological age, for very long periods. Juvenile copies of the germplasm can be maintained while clonal material is field tested on a large-scale, and elite clones can subsequently be selected and used for advanced breeding programmes and commercial forestry. The development of this technology for the propagation of elite material of radiata pine in a commercial environment will be described.

Corresponding author : Carolyn Raymond

Raymond , Carolyn[1*]

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Title : GENETICS OF EUCALYTUS WOOD PROPERTIES

Abstract : Traditional methods for assessing wood properties were both destructive and expensive, limiting the numbers of samples that could be processed. Over the past decade, non-destructive sampling techniques and new assessment methods have been developed leading to a large increase in the numbers of trees and traits that could be evaluated. This technology has enabled the assessment of progeny trials to determine the patterns of variation, degree of genetic control and economic importance of many wood traits, leading to the inclusion of wood properties in many eucalypt breeding programs. Issues addressed in this paper include what are the potential markets and products for plantation eucalypts, which wood properties should be assessed, sampling and assessment methods and genetic control and patterns of variation for a range of wood properties.

Corresponding author : Rupert Wimmer

Rupert, Wimmer*

University of Agricultural Sciences, Vienna, Austria

*indicates the presenting author(s)

Title: WEATHER, WATER, WOOD: ENVIRONMENTAL EFFECTS ON WOOD AND FIBRE PROPERTIES

Abstract: Wood formation is a dynamic physiological process, and any changes in the immediate environment in which the tree is growing can affect the growth and thus its pattern of growth and wood properties. An understanding of the factors influencing wood property formation in the cambium at a sub-annual level can only be drawn if the growth process and conditions can be linked to the formed wood. Results from recent research show wood properties (wood density, microfibril angle) being observed at daily time steps during wood formation. It is demonstrated how irrigation, soil water deficit, temperature and other factors influence wood formation of trees which helps to improve our understanding of how wood quality is determined.

Corresponding author : Daniel Carraway

Daniel, Carraway*
ARBORGEN
*indicates the presenting author(s)

Title: INDUSTRIAL PERSPECTIVES AND APPLICATION OF BIOTECHNOLOGY FOR SUSTAINABLE PRODUCTION OF HIGH QUALITY WOOD.

Approximately forty percent of the world's timber supply currently comes from natural forests. The 'hunter-gatherer' analogy has frequently been applied to the process of supplying wood to manufacturing facilities from natural forests. Plantation forestry can enable wood supply efforts to move from a hunter-gatherer model toward an agricultural model. Application of biotechnology to plantation forestry will increasingly enable access to sustainably produced fiber at economically feasible prices thereby reducing pressure on natural forests. Biotechnology and plantation forestry will also become increasingly important to offset the impact of carbon emitting industries on global environmental health by serving as carbon sinks. Forest tree improvement practices, including biotechnology, play a key role in improving the quality, productivity and economics of wood supply in the context of plantation forestry.
Application of biotechnology to the practice of plantation forestry could enable higher growth rates coupled with greater fiber uniformity and quality, better cost predictability and control, and more resource management options. In addition to the economic benefits, environmental benefits are expected from application of biotechnology to plantation forestry. Using biotechnology to help supply the growing world demand for wood can stabilize or improve the health of forest ecosystems by improved conservation and/or species and landscape restoration.Organizations that engage in the responsible practice of biotechnology will be catalytic, enabling, educational, international, and community builders. Industry, private research institutions, and universities must develop extensive collaborations to ensure sensible regulation and public support for the application of biotechnology if the world's economy and environment are to benefit.

Corresponding author : Herman Höfte

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Title : ARABIDOPSIS AND CANDIDATE GENES FOR WOOD QUALITY QTLS IN MARITIME PINE.

Abstract : Technological properties of wood products are to a large extent under genetic control. One major challenge is to develop reliable molecular markers that can be used to select, at a seedling stage, those genotypes that will lead to trees producing wood of superior quality. QTLs for a number of parameters relevant for wood quality have been mapped in maritime pine. In order to find molecular markers that are in a linkage disequilibrium with favorable alleles in natural populations, the genes corresponding to the QTLs need to be identified. Given the size of the genome, a candidate gene approach is the only realistic way to identify those genes. The strategy of the EEC-funded GEMINI project is to identify candidate genes and to validate them by sequencing corresponding alleles in natural populations. Screening for transcripts differentially expressed during the development of wood of different properties on one hand, and mapping of the corresponding genes on the other hand, leads to a large number of candidate genes for the QTLs. The major bottleneck in this approach is the validation of these genes. The choice of the appropriate candidate genes for further study requires an intimate knowledge of the biological processes underlying the parameter under consideration. Arabidopsis is the ideal model for the molecular dissection of these processes, as has been shown in the past for instance by the study of the biosynthetic pathway of monolignols. Similar studies are now providing more insights into cellulose biosynthesis, the control of cell size, the orientation of the microfibrils etc. In addition, reverse genetics can be used to rapidly analyse the function of Arabidopsis orthologs of novel pine genes with interesting expression patterns located in QTL intervals. Some examples will be discussed.

Corresponding author : Björn Sundberg

Björn Sundberg[1*], Anders Andersson[2], Henrik Aspeborg[2], Rupali Bhalerao[3], Kristina Blomqvist[2], Magnus Hertzberg[1], Joakim Lundeberg[2], Ewa Mellerowicz[1], Peter Nilsson[2], Göran Sandberg[1], Jarmo Schrader[1], Tuula Teeri[2], Mathias Uhlén[2]

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Title: EST SEQUENCING, MICROARRAY ANALYSIS AND WOOD FORMATION IN POPLAR

Abstract: The value of poplar as a model tree species has increased significantly with the Swedish initiative to produce large scale expressed sequence tag (EST) libraries. EST databases have been created from different organs and a variety of tissues and environmental conditions. Up to March 2001 about 35.000 EST sequences have been produced, covering approximately 10.000 individual gene sequences. An initial set of 3.000 ESTs from the wood forming tissues has been prepared to produce a poplar microarray for the analysis of global gene expression pattern. The array was used to construct a transcriptional roadmap to wood formation. mRNA prepared from narrow wood developmental zones obtained by tangential cryosectioning was used to probe the microarray thus revealing global gene expression changes from cell division in the vascular cambium to late fiber maturation. The analysis demonstrated a strict developmental regulation of many genes including genes encoding enzymes involved in biosynthesis of lignin, cellulose and other cell wall components. The high spatial resolution of expression patterns that was obtained with our technique reveals information on gene function in xylogenesis. EST databases combined with microarray data are used to identify candidate genes, with both known and unknown function, for the regulation of wood formation. 60% of the arrayed genes have high similarity to Arabidopsis genes. Of these, 498 have their best hits to Arabidopsis genes of unknown function. Now, the role of these genes in secondary xylem development can be tested both in Arabidopsis and in poplar.

SESSION 2

Chairs, Colin Matheson/Helena Pereira

Methods for the assessment of wood and fibre properties. Modelling wood properties at different scales

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Title : RAPID MEASUREMENT OF THE TRUNK EQUIVALENT MODULUS OF ELASTICITY ON STANDING TREES WITH THE RIGIDIMETER

Abstract : A new device has been developed in order to determine experimentally the modulus of elasticity of standing trees: the Rigidimeter. It has been used on a retrospective hybrid Larch clonal test located at INRA Orléans France. Some of the trees were felled and studied. The objectives of the study are to contribute to the validation of the Rigidimeter as a fast, non-destructive, reliable tool for rapid evaluation of an important wood mechanical property; the goal of this contribution is to evaluate the influence of 1) the measurement method of tree diameter, 2) the presence of bark, 3) some details of the mesurement procedure on the precision of the estimation of the trunk modulus of elasticity.

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Title : SUCCESSFUL ACOUSTIC SEGREGATION OF PINUS RADIATA LOGS ACCORDING TO STIFFNESS

Abstract : Wood quality at harvest-age varies significantly both within and between Pinus radiata trees, leading to a wide range in sawlog quality. It is highly desirable to be able to be able to sort logs precisely, either in the field or the log yard, into the specific grades established by a pre-harvest inventory (e.g., MARVL). Currently, visual assessments are used to grade logs. It is known that visual grading procedures do not accurately reflect the intrinsic quality and Modulus of Elasticity (MoE) of the wood, and the performance of the structural products sawn. It is known that Non Destructive Evaluation (NDE) tools are able to predict wood stiffness and strength reasonably well. Thus, the incorporation of NDE tools with visual grade assessments will ensure that predominantly high quality logs are processed by saw and veneer mills. This study examined whether acoustic sound flight velocity (m/s) along with other NDE tools could be used as a direct measure of wood stiffness for log grading. Mature trees of radiata pine were measured before and after harvest with non-destructive devices to examine the relationship between sound wave velocity and density in either standing trees or logs and the MOE of clearwood axial specimens and machine stress graded boards. The speed of sound along logs was sufficiently closely correlated with wood stiffness to allow logs to be sorted into classes. A highly significant and positive relationship was found for acoustic measurements made in logs and a weaker, but still significant, relationship existed for acoustic measurements made in standing trees. Acoustic tools may also be used as an indirect tool for selection provided the heritability of the measurements is high enough and there is significant genetic correlation with genetic values for wood stiffness. From another experiment we have estimated the heritability of several acoustic measures and hope to be able to estimate genetic relationships with wood quality soon.

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Title : USING VIBRATION ANALYSIS FOR QUALITY CONTROL ALONG THE WOOD CHAIN: FROM THE LOG TO THE END PRODUCT

Abstract : Non destructive evaluation of mechanical properties is one of the key issues for an optimised wood chain, from the forest to the building industry. Analysing the natural vibration freqencies of timber is a promising method for the assessment of its elastic properties, and potentially the way they vary within the piece of timber (detection of heterogeneity). This method has been successfully applied to beams, reconstituted beams, and more recently wood based panels. In the latter case, it is possible to evaluate the stiffness matrix in the plane of the board, and this constitute an efficient quality control method. New investigations concern the non destructive evaluation (NDE) of logs characteristics, and especially logs that have been stored under wet conditions. A comparative analysis of different NDE techniques, including ultrasonic measurements, applied at different stages of log processing, is presented in this paper. The results show that vibration analysis appears to be a powerful method.

Corresponding author : AHMED KOUBAA

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Title : TRANSITION FROM EARLYWOOD TO LATEWOOD IN BLACK SPRUCE FROM X-RAY DENSITOMETRY DATA

Abstract : Estimating the transition from earlywood to latewood in annual rings is an important task since the accuracy of several density and growth components depends on this estimation. Mork's index has long been used as an anatomical definition of the transition from earlywood to latewood in annual rings. This definition is arbitrary and the index is extremely difficult to measure and its application in X-ray densitometry is impossible, unless the density is measured on microscopic wood samples. In X-ray densitometry, a threshold density of between 0.40 to 0.55, depending on the wood species, is usually chosen to differentiate between earlywood and latewood density. This method can introduce important errors in estimating features such as earlywood and latewood densities and widths and latewood proportion. Consequently, new approaches should be developed and integrated in the computational programs used to generate X-ray data. In this study, we present a mathematical approach. We modeled the within ring pattern of variation of wood density in 100 plantation grown black spruce trees using high order polynomials. The fitting between observed and measured densities is excellent since the coefficient of determination is well above 90%. We defined the transition from earlywood to latewood as the inflexion point. Results indicated that the earlywood to latewood transition varies from juvenile to mature wood. The presented method could be easily integrated in any X-ray densitometry program and it allows comparison among rings in a consistent manner. This method is promising for work related to the genetic variation of earlywood and latewood densities and widths.

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Title: EFFICIENT WOOD AND FIBER CHARACTERIZATION- A KEY FACTOR IN RESEARCH AND OPERATION

Abstract: During the last years, a tremendous development has taken place in biotechnology. In the field of tree improvement, new methods have increased the knowledge base, links to wood properties have been investigated and applications are now approached. When entering the phase of industrial application, the new possibilities have to be combined with knowledge about the demands of the "user" concerning product quality, production efficiency, etc. It is, for instance, important to increase the volume production of pulpwood per hectare, but if this is achieved trough the formation of fibers and vessel elements with changed properties, the pulpwood may not be suitable for the same product. It is also crucial to establish knowledge about the natural variability of the wild-type plants and about age-to-age relationships. Otherwise, it is not possible to predict from young plants if the new material will be better at a suitable age. Methods for characterization are key factors for progress in this field, in research as well as in operation. The methods have to be efficient to allow investigation of large enough sets of samples for representative results.

At STFI, the Swedish Pulp and Paper Research Institute, new tools and knowledge for improved wood and fiber utilization have been developed, within national and international research projects and contract work. Characterization of wood and fiber properties has been emphazised. In this presentation, examples will be given from such measurements. One set of examples will show results from measurements of growth structure, wood basic density, moisture content, heartwood, earlywood, latewood contents and fiber dimensions. The data will be visualized as maps on the variability within whole trees. These examples originate from the project EuroFiber within the Fifth Framework Programme of the European Commission. The EuroFiber project, coordinated by STFI, includes raw material characterization as well as experiments on laboratory, pilot plant and mill scales. Another example will show characterization of individual fibers and vessel elements in wood and illustrate age-to-age relationships. It originates from the POPWOOD project funded by the same EC programme and coordinated by CNRS/ISV, including genetic tree improvement and evaluation of the resulting materials as a raw material for pulp and paper.

Other research areas at STFI in this field are development of data on variability in wood and fiber properties, models for variations within and between trees, stands and regions and relationships between raw material and product properties. With this arsenal of tools and knowledge, STFI is looking forward to make further contributions in new research projects.

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Title : ENGINEERED WOOD PRODUCTS FOR CONSTRUCTION: QUALITY REQUIREMENTS

Abstract : This paper reviews the mechanical properties of a range of engineered wood products (EWP) used in construction, from glued laminated timber to structural boards and derived products, such as I-joists. The principles of reconstitution in EWP, and its effect on the performances of such products, are presented in the first section of the article. The mechanical behavior of glued joints is one determinant of the reliability of structural composite materials, and is discussed on a theoretical basis supported by experimental data. The main factors affecting the properties of such products are analyzed in a second part, including: size effects, duration of load, stress concentration near defects or geometric singularities. Requirements on raw material quality, including genetic improvement, are discussed in the last section.

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Title : THE INSTRUMENTED MICROTOME CUTTING TESTS ON WOOD FROM TRANSGENIC TOBACCO AND POPLAR PLANTS WITH MODIFIED LIGNIFICATION

Abstract : Reducing the amount of lignin, or modifying its architecture, can bring benefits in pulping, but it is important to understand the relationship between levels and types of lignification in plants and their effects on mechanical properties. As part of an EU funded tree improvement project (TIMBER), this paper summarised our instrumented microtome cutting (IMC) test results on genetically engineered tobacco and poplar plants. The results show that control plants have greater cutting work than transgenic lines. This indicates that lignin modification has changed the fracture properties of the lignified tissues. Keywords: lignification, transgenic plants, wood, cutting work.

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Title : VISUALISATION OF WOOD MICROSTRUCTURE USING SILVISCAN

Abstract : SilviScan is a set of automated instruments designed for the rapid assessment of wood microstructure in samples cut from increment cores. Many thousands of samples are measured per year, each run producing Gbytes of data. Therefore data manipulation and storage is a major concern. Relevant information must be extracted and the majority of the raw data deleted. Although automation of this process is essential, it is difficult to develop robust algorithms without an appreciation of the information content of the data. The ability to understand complex models and to recognize key information in large, often multidimensional, data sets can be greatly enhanced by presentation in the form of images. This report illustrates the use of images and graphs to visualise SilviScan data. A selection of images is shown, pertaining to the three major functions of the system: optical microscopy, scanning x-ray microdensitometry, and scanning x-ray diffractometry. Examples of the spatial variation of wood properties within trees are also given.

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Title : RELATIONSHIP BETWEEN RESIN ACID CONTENT AND ELECTRICAL PROPERTIES OF SCOTS PINE WOOD

Abstract : It is known that the wood extractives such as resin acids have an effect on the decay resistance of wood. If the resin acid content and the electrical properties have a relationship, an electrical NDT-method might be used to predict the decay resistance of living trees. This study examined the relation between resin acid content and the electrical properties of wood specimens at three controlled high moisture conditions (RH 97% and two wetting experiments). All specimens had the same moisture treatments before the electrical impedance measurements. Complex impedance was measured from heartwood and sapwood specimens using frequencies between 50 Hz and 5 MHz. The samples of Scots pine heartwood were obtained from 34-year-old trees, and the samples of pine sapwood from 7-year-old seedlings. Resin acids were extracted from powdered wood samples with petroleum ether-diethyl ether. The extracts were analysed by gas chromatography - mass spectrometry. Both sapwood and heartwood sets gave same type of results: specimens with high resin acid content had lower electrical capacitance and higher electrical resistance as compared to the specimens with low resin acid content.

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Title : MODELLING AND SIMULATION OF TREE AND STAND GROWTH : APPLICATION TO TREE BIOMECHANICS

Abstract: Over the last ten years, the AMAP Plant Modelling Program (CIRAD, Montpellier, France) has developed high technology computer tools for analysing, modelling and simulating tree growth, structure and function. The description of the dynamic development of plants is based on a vast amount of knowledge accumulated from many different disciplines, including botany, physiology and mathematics. Simulation programs allow virtual 3D plants and trees to be represented at different ages throughout the plant's life.

Recently, new models have been developed to simulate individual tree growth, whilst also incorporating mechanics into these models. This work aims to investigate both mechanical stability and wood quality with regards to tree growth. The model is based on a mathematical description of plant architecture, which can automatically be altered, depending on the tree's local environment e.g. competition for space and light or a response to gravitropism. A particular function in the model allows the mechanical behaviour of trees to be taken into consideration. This mechanical calculation focuses on the relationships between the structure and shape of the stem, introducing righting movement processes due to cell maturation, in both normal and reaction wood. The evolution of stem form according to the equilibrium of the tree can thus be simulated and the 3D distribution of growth stresses occurring inside the trunk calculated.

More recently, some developments have been carried out in order to incorporate these biomechanical concepts into growth models at the stand level (software CAPSIS, INRA, France). This approach allows different sylvicultural scenarios to be taken into consideration.

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Title : NEAR INFRARED ANALYSIS AS A RAPID SCREENING TOOL FOR SOME MAJOR WOOD CHARACTERISTICS IN EUCALYPTUS.

Abstract : The ability to assess wood quality is of primary importance to the forest industry. In the case of intensive management forest such as clonal plantations of Eucalyptus where the heterogeneity of the raw material is huge, it is fundamental to be able to predict whole-tree values for wood properties from non-destructive sampling techniques. Such prediction for selection purposes is currently hampered by costly and time-consuming traditional chemical and technological assays. One of the methods most likely to be adaptable to rapid measurements on wood is Near Infrared diffuse reflectance spectroscopy (NIRS). NIRS spectroscopy is known for its good ability to identify compounds as it is mentioned in a lot of publications. This paper evaluated the ability of NIRS for the assessment of some chemical, physical and mechanical wood characteristics within a full-sib family of a hybrid Eucalyptus. Quantitative relations between NIR spectral bands and extractives content, lignin composition, Surface Longitudinal Growth Strain (LGS), shrinkages and modulus of elasticity are analysed in terms of prediction accuracy. In order to evaluate the ability of the NIRS method to be a technique for a rapid assessment of the above properties different sampling strategies are evaluated : ground (extractives free or not) and solid wood samples are compared. Moreover the use of a fibre optics module on NIRS system as a practical sensor for solid wood measurement is also tested.

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Title : RAPID NON DESTRUCTIVE METHODS FOR ASSESSMENT OF WOOD CHEMICAL COMPOSITION

Abstract : Wood chemical composition is an important parameter to assess wood properties, especially when it is used as raw material for chemical processing as it is the case in the pulping industry. The traditional wet lab chemical methods are not suitable for analysing the wood chemical composition of a large number of samples, as is often necessary in tree breeding programs, due to the fact that they are time consuming and expensive. Recent developments of indirect methods to assess lignin and polysaccharide composition by spectroscopic techniques as well as lignin quality by analytical pyrolysis may provide the answer to overcome the problems associated to wet chemical methods. In this presentation we will make a review of the main methods in development for rapid non destructive wood analysis (FTIR, NIR, Raman and analytical pyrolysis) and we will discuss the merits of each one, with some case studies related to Eucalyptus globulus, Pinus pinaster and Picea abies.

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Title : KRAFT PULPING AND HEARTWOOD DEVELOPMENT IN MARITIME PINE

Abstract : The presence of heartwood in the raw material used for pulping decreases pulp yield and brightness mainly as a result of a higher content of extractives in relation to sapwood. In spite of the impact of heartwood on pulping, research is scarce for species used for pulping, i.e. very little is known regarding maritime pine, an important species for kraft pulping in Portugal, Spain and France. The within tree development of heartwood was evaluated in 75 year-old maritime pine trees (Pinus pinaster Ait.). At 1.3 m height, heartwood represented 17% of the cross-sectional area, extending to 17 annual rings and sapwood contained 54 annual rings. Heartwood decreased along the tree and at 65% of total tree height it represented 5% of the cross-section with. The content of extractives differed largely between heartwood and sapwood, with respectively 19.7% and 5.8% on average. In a cross-section, the pulp yield of heartwood was always lower than the pulp yield obtained with sapwood: on average 40.0% for heartwood and 49.7% for sapwood for similar delignification degree (kappa number). Pulp yield was negatively correlated with the content of polar extractives (ethanol and water solubles).

SESSION 3

Chairs, Francisco Zamudio/ Bo Karlsson

Natural variation and quantitative genetics of wood and fibre properties. Connection between sylviculture and wood properties

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Title : EFFECT OF INBREEDING ON WOOD DENSITY IN RADIATA PINE

Abstract : Effects of inbreeding on basic wood density in a 17-year-old radiata pine trial were studied using five populations each inbred to one of five inbreeding levels: outcross (F=0), half-sib (F=0.125), full-sib (F=0.25), selfing (S1, F=0.5) and two-generations of selfing (S2, F=0.75). These five populations were derived from a founder population of eight clones. Inbreeding had no significant effect on basic wood density although inbreeding slightly depressed wood density (inbreeding depression was 1.47%, 2.50%, 1.65%, 0.02%, respectively at F=0.125, 0.25, 0.50 and 0.75). However, inbreeding increased variation of wood density among trees by 3.70%, 3.40%, 15.74%, and 29.01%, respectively, for population at F=0.125, 0.25, 0.5, and 0.75. The basic wood density at about 400 kg m^3 was increased from age 4 (earliest age for most samples) to about age 11 and 12 and stabilized thereafter in all five populations around 525 kg m^3. Non-significant inbreeding depression on wood density at population level and higher variation of wood density in the inbred populations may indicate that inbred lines for higher wood density could be derived quickly through inbreeding approach. The low inbreeding depression in radiata pine for growth and no inbreeding depression for wood density have rendered radiata pine as an ideal species to use inbreeding as breeding tool.

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Title : NATURAL AND GENETIC VARIATION OF WOOD DENSITY IN LARIX SPP. GROWN IN SIBERIAN TAIGA

Abstract : Larch (Larix Mill.) is one of the most important elements of the boreal forests. The widest distribution of larch in the world is to be found in Russia. The wide genetic variation of larch species in Russia has, to a great extent, not earlier been available for forest research in western Europe. The objective of the paper is to study the natural variation in wood density of the three larch species within Russia, *Larix sukaczewii* Dyl., *L. sibirica* Ledeb., and *L. gmelinii* Rupr.. Increment cores were collected from 386 individual trees distributed over eight regions (*L. sukaczewii*: 134 trees from 3 regions, *L. sibirica*: 153 trees from 3 regions and *L. gmelinii*: 99 trees from 2 regions). Wood density and ring width were determined by soft-X-ray densitometry. Preliminary analysis of data showed that there were significant differences between regions on wood density. We will discuss the details of these differences from species differences point of view.

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Title : GENETIC VARIATION IN WOOD SPECIFIC GRAVITY OF HALF-SIB FAMILIES OF PINUS NIGRA SUBSP PALLASIANA: IMPLICATIONS FOR EARLY SELECTION

Abstract : Seedlings from 7 populations (total of 281 half-sib families) and 35 seed stands representing natural range of Anatolian black pine (Pinus nigra subsp. pallasiana) were planted in a forest nursery in Ankara in 1990 and raised until age 3. Wood specific gravity of stem woods of 3 year old seedlings from half sibs families and seed stands were determined. The results of this study indicated that WSG did not vary significantly neither between populations (ranging from 0.41 to 0.42) nor between 35 seed stands (WSG ranged from 0.371±0.04 to 0.456±0.07). Differences between half sib families for WSG were, however, statistically significant. Estimated family heritablity was moderately high (0.38), indicating that early selection of families for WSG would be possible and substantial genetic gain in WSG will be achieved if Anatolian black pine plantations are made with families with high WSG. Genetic correlations between seedling growth traits and WSG were low, but consistently negative. The families with more height and diameter growth had lower WSG values. Also families with late budset and budburst dates in 1991 had lower WSG values. Seedlings originated from northern latitudes had lower WSG than those from southern latitudes.

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Title: FOREST TREE BREEDING AND BIOTECHNOLOGY APPLICATIONS IN UKRAINE

A regular research activity in forest tree breeding started in Ukraine since 30^{ths}. A big experience in this area has been gained in Ukrainian forest research institutions during the time spent. The most considerable results have been obtained in investigations of forest tree species diversity, gene resource conservation, tree improvement, intra- and interspecific hybridization, etc.
Investigations on microclonal propagation and biotechnology of agricultural plants started in Ukraine in early 80 ^{ths}. Activity on applying microclonal techniques in forest tree breeding started in late 80 ^{ths}. Several national projects were launched in middle 90^{ths}. Target species: hybrid poplars, *Quercus robur*, *Juglans regia* and its hybrids, other hardwood species. Two international projects started in 2001. Biotechnology researches are a part of one of them. Conservation of gene resources of broadleaf tree species including *it vitro* techniques and a EUREKA Strategic Project EUROFOREST (2144 "New technologies for the tree and the green areas") and biotechnologies.

Projects:
Lviv – microclonal propagation of poplars for land reclamation
Kharkiv – microclonal propagation of forest tree species for tree breeding
Kharkiv – microclonal propagation of *Quercus robur* for gene conservation (Luxembourg)
Kharkiv + Institute of Gene Engeneering (Kyiv) – microclonal propagation of hardwood species

EUROFOREST's main goal is the development of new technologies aimed at improving sustainable management in the european forestry sector and the green areas.

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Title : PREDICTION MODELS FOR WOOD PROPERTIES OF NORWAY SPRUCE AND SCOTS PINE IN SWEDEN

Abstract: Wood properties are of general interest to improve process efficiency and better wood and fiber based products. To mapping and modelling the variation in basic wood properties, a structured sampling procedure including the expected main sources of variation was carried out within the frames of the Forest-Pulp-Paper project in Sweden. Totally 252 sample trees from 42 Norway spruce stands and 120 sample trees from 20 Scots pine stands were selected according to differences in climatic conditions (Latitude 56.6 – 65.8°, altitude 60-440 m), site indices, stand maturation and tree size. Plot and tree measurements were followed by a wood sampling from different heights in each sample tree. Basic density, contents of latewood, juvenile wood and heartwood and bark thickness were measured. Mixed linear model attempts have been tested based on diameters, number of annual rings and climatic indices (Temperature sums in day-degrees) as the only explanatory variables. Between 49% (Spruce basic density) and 94% (Spruce heartwood diameter) of the total variation was explained by these variables while a considerable part of the residual variance was explained by between tree variance. A minor part of the remainder was explained by random between stand variance. The presented models may be used for wood property predictions in forest planning as well as for operative bucking and property predictions in the harvesters. The models have been validated on independent data from different earlier studies of wood properties including ordinary forest stands and genetic field tests. Results from validation harmonise with estimated prediction errors and variance components based on data used for model calibration.

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Title: GENETIC PARAMETERS OF WOOD PROPERTIES IN *PICEA ABIES*

Abstract: Genetic parameters were estimated for several wood and growth traits in two 19-year old clonal trials and a 40-year old full-sib progeny trial of *Picea abies* (Norway spruce) in southern Sweden. The work was carried out within the EU-project GENIALITY. In the clonal trials high broad sense heritabilities (H^2>0.4) were observed for wood density traits, lignin content, number of internal cracks, growth traits, spiral-grain angle and number of resin canals. Medium heritabilities (0.2<H^2<0.4) were observed for tracheid lumen diameter, microfibril angle and tracheid length, and low heritabilities (H^2<0.2) were found for cell-wall thickness, pulp yield, FS-factor (zero span measure of the strength of individual fibres), wood stiffness and wood colour. Narrow sense heritabilities estimated from the progeny trial for the corresponding traits tended to fall into the same range as broad sense heritabilities, indicating negligible non-additive effects for these traits. Exceptions were lignin content, spiral-grain angle and growth traits, which showed lower values in the progeny trial. The genotypic coefficient of variation showed normal levels (5-15%) for most of the traits in the clonal trials. The traits with least genotypic variation were lignin content, the wood colour traits and pulp yield, all traits with a genotypic coefficient of variation below 2.2%. Most traits showed low levels of genotype by environment interaction as indicated by genotypic correlations between sites close to or above 0.7. The most stable traits were wood density, spiral-grain angle and number of internal cracks. Least stable were pulp yield (r_G=0.43) and cell-wall thickness (r_G =-0.19). Among the wood properties, only number of resin canals, latewood proportion, earlywood density and ring density showed significant negative correlations with volume in both clonal trials. The three latter traits all showed strong negative genotypic correlations (r_G<-0.7) with volume. The implications of these findings for the breeding of *Picea abies* are discussed.

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Title : GENETIC PARAMETERS FOR SPIRAL-GRAIN ANGLE IN TWO 19-YEAR-OLD CLONAL TRIALS WITH *PICEA ABIES*

Abstract : Spiral-grain angle was measured for all year rings on wood discs from two 19-year old (field age) *Picea abies* clonal trials. In both trials, mean grain angle reached a maximum inclination to the left at ring number 4, followed by a monotonic decrease towards a right-handed inclination. Clonal means of mean grain angle of ring 3 to 15 ranged from 0.5 to 4.7 degrees and from -0.2 to 5.3 degrees in the two trials, respectively. The broad sense heritability of mean grain angle were in both trials high (H^2=0.42) whereas the heritabilities of individual rings generally were slightly lower. The slope of the radial grain development showed moderate to high heritability in the two trials and negative genotypic correlations with grain angle in the year rings close to the pith indicating that clones with high grain angle in the inner rings tended to have a more rapid development towards a straight angle in the following rings. A selection based on any of the year rings in the interval of ring number 5 to 10 was most efficient in decreasing the mean grain angle of ring number 3 to 15.

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Title : GENETIC VARIATION OF WOOD DENSITY RECORD OF CAMBIUM REACTION TO WATER DEFICIT IN PICEA ABIES

Abstract : Breeding for adaptation is generally the first and most important goal of forest tree improvement programs. Adapted trees are trees that are physiologically suited for high survival, good growth and resistance to pests and adverse. It is thus very important for the tree breeder to be able to estimate genetic variability of the tree response to pest and adverse conditions. The product of tree growth is wood. Under temperate climates, wood formation is periodic. The aggregation of successive rings designs the tree stem. Each ring is made of earlywood and latewood. Water availability during the growing season is one of the main constraint for tree growth For different softwood species, a water deficit during the first part of the growing season results in the formation of a "false ring": latewood-like cells located in the earlywood. A density peak in the earlywood can thus be understood as a record of the cambium reaction to an adverse condition, such as a water deficit. Such a density peak was observed in the earlywood of most Picea abies trees from a clonal test in southern Sweden. We measured some simple characteristics of the density peak in the earlywood of the ring formed in 1993 in 20 Picea abies clones located in 2 experimental sites in southern Sweden. We found a highly significant clonal variation for all the peak characteristics. The most variable characteristic was the peak location in the ring. This trait was also extremely variable between sites. There was mostly no site effect for the other peak characteristics, et mostly no strong or not significant site-clone interaction for the peak characteristics. The consequences of the clonal variability of the tree response to the 1993 water deficit on clones radial growth and ring density is presented and discussed.

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Title : GENETIC SELECTION WITHIN EUROPEAN DOUGLAS FIR FOR PAPERMAKING USES

Within the frame of the EUDIREC European project (FAIR CT 95-0909), a programme has been built to help breeders to choose, in the future, among all the wood characteristics, those fairly easy to collect directly on trees in field tests or non destructive samples, and those showing the highest genetic gains in relationship with industrial uses of Douglas fir wood.
The interactions between the TMP process and the wood properties were studied on a sample of thirty trees 17 year old, using a specific procedure on a 12'' Andritz refiner. From a multivariate analysis, it was concluded that physical properties of the TMP hand sheets are firstly linked with anatomical parameters like [Cell wall thickness / lumen diameter], but also with within ring density related traits calculated from the X ray profile. In particular the combination of the average wood density and the number of intersections between the 700 g / dm^3 density threshold and the density profile can help breeders in screening wood samples for TMP strength. Initial brightness of TMP handsheets is correlated with wood colour (a* chromatic component of wood on LT surface), but bleachability could not be predicted from the evaluated parameters. Lignin, holocellulose and extractives content were considered for predicting the Kraft pulping yield. Lignin and holocellulose contents can be estimated directly on non extracted wood powder through near infra-red spectroscopy.
16 clones out of 200 were selected within a 24 year old test (Kattenbuhl, Germany), using the previous wood predictors in order to evaluate the range of the clonal variability for the papermaking potential. The genetic control of the physical and mechanical wood properties is obvious and chemical analyses give also evidence of samples exhibiting different holocellulose content (for the same lignin content) The within clone variance is much higher for the extractives content than for the lignin and cellulose content, which can explain the rather weak discrimination of genetic units for the Kraft pulping yield in spite of differences between units of more than 4 points. The clone discrimination of the fibre length is weak, to the difference with the coarseness as a consequence of the huge clonal variability of the latewood density levels. The industrial selection gain for pulping is discussed after testing average wood assortments on a TMP pilot plant (physical, optical properties after bleaching, CDO, energy consumption) and leads to general recommendations for breeders and the pulping industry.

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Title : EFFECT OF GROWTH RATE ON THE FIBRE PROPERTIES OF NORWAY SPRUCE (PICEA ABIES (L). KARST.)

Abstract : The purpose of this study was to determine the effects of growth rate on microfibril angle (MFA) and fibre dimensions of Norway spruce. Material for the study was sampled from a nutrient optimisation experiment in Flakaliden, northern Sweden (64°07' N, 19°27' E, alt. 310 m). The experiment was established in 1987 in a 28-years old stand. The 'optimal' nutritional status was defined in terms of target needle concentrations for each individual nutrient element. Fibre length and diameter increased rapidly from the pith outwards. Fast growing fertilised trees had remarkably shorter fibres than control trees. Also, fast growing fertilised trees had wider fibres than the control trees. However, the relationship between fibre diameter and distance from the pith was similar in the fertilised and control trees. In addition, fertilisation reduced fibre wall thickness and yearlywood and latewood density. Fertilisation increases growth of Norway spruce, but in the same time, affects wood properties. Fibres get wider and cell walls thinner. On the other hand, MFA does not seem to change as a result of fertilisation. However, the differences in fibre properties were less apparent when they were examined with regards to distance from the pith. Therefore, fibre dimensions are rather determined by the number of anticlinal cell divisions taking place in the cambium than by cambium age.

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Title: GENETIC VARIATION IN WOOD DENSITY THROUGH CAMBIAL AGE IN A RADIATA PINE PROGENY TEST AND ITS RELATIONSHIP WITH RADIAL GROWTH

Abstract. Genetic variation in wood density through cambial age in a radiata pine progeny test and its relationship with radial growth. Objectives of the analyses reported here were to evaluate the genetic variation of ring density through cambial age and to study the ring-ring genetic relationship between wood density and radial growth. Wood samples from 31 half-sibs families of radiata pine established in a progeny test in Chile were obtained and submitted to a X-ray densitometry procedure. The analyzed traits were total ring width (TRW), ring area (RA), and average ring density (ARD). Statistical analyses were conducted to determine heritability at the ring level and ring-to-ring genetic correlation. Trait-to-trait genetic correlations between ARD and radial growth were also measured. ARD recorded a strong genetic control at the first rings, a weak control between rings 5-8, and a moderate control after ring 9. Ring-ring genetic correlation were very high for every character. The transition zone between juvenile-mature wood had a strong influence in the pattern of change of genetic parameters.

SESSION 4

Chairs, Dave Cown/ Guillaume Chantre

Wood and fibre properties for particular end uses

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Title: INDUSTRY RESPONSE TO CHANGING WOOD QUALITY NEEDS

Abstract: New Zealand is a country in the southern hemisphere, well known for the development of extensive temperate pine forests to counter the depletion of natural forests and the stabilisation of agricultural land. The current (and rapidly expanding) forest production is much in excess of local demand, and the need to seek export markets, together with globalisation of trade, has caused dramatic changes in forest operations and consequently wood quality.

The predominant species (*Pinus radiata*) is very pliant silviculturally and produces a versatile wood, capable of meeting a wide range of customer specifications. However, variability, both within and between stems, coupled with a reduced rotation age and a higher proportion of juvenile wood, are increasingly critical issues. Wood processors are placing increasingly stringent limits on the types of logs and lumber quality that they will accept in their operations. Forest growers have responded by adopting more sophisticated methods for determining wood quality in plantations and segregating material into narrower quality bands. While log grading based on external characteristics has been a feature of domestic and export markets for some years, several new technologies are being introduced to ensure uniformity in internal wood properties of material currently being harvested.

The paper will discuss current industry approaches to two juvenile wood issues – stiffness and internal checking. Research has already demonstrated the relatively high heritability of a range of important wood properties. For the longer term, progressive forest companies have collectively invested in research to identify the most likely future markets and key wood properties requiring improvement.

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Title : EFFECT OF DRYING TREATMENTS ON WARPING OF WHITE SPRUCE PROVENANCES

Abstract : Sustainable forest management is now strongly promoted by most of the countries all around the world. Furthermore, in Canada there is a growing consensus for increasing the proportion of the natural forests set apart for conservation and non timber activities. Consequently, wood from plantation is becoming more and more a major source of supply for the lumber industry. White spruce (Picea glauca [Moench] Voss) is one of the main reforestation species in Canada. Each year, between 15 000 and 20 000 hectares are reforested with this species in the province of Québec only. Due to tending operations and use of fast-growing and shorter rotation stocks, wood from plantation is likely to possess properties that are different from that presently used by the industry. It could, therefore, require costly adjustments to the manufacturing processes. In this study we investigated the genetic variation in warping in the kiln drying of 26 white spruce provenances from the Great Lakes and St. Lawrence region. Two drying treatments were applied, i.e. conventional and high-temperature drying. For bow, crook and twist defects after drying, no significant differences were found among the provenances tested. However, significant differences were revealed between the average of all provenances (plantation-grown) and wood from second-growth forest for crook and twist defects. Results are presented and discussed.

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Title : STRUCTURAL LUMBER OF FAST-GROWN NEW ZEALAND RADIATA PINE: PILOT COMPARISON OF CLONES, CP FAMILIES AND CLIMBING SELECT (OP) AT AGE 11-13.

Abstract : Since the first 10 rings comprise nearly half of the merchantable volume of an age-25 radiata pine stem, poor outturn of structural lumber from corewood will limit sawmill profits. This study involved 16 trees of an open-pollinated seedlot (age 13), 8 trees each of 18 control-pollinated families mainly involving top orchard parents (age 13), and 6 trees each of 3 micropropagated clones (age 11). Two flat-sawn kiln-dried boards (2.4m x 100 x 40 mm) were produced from the outerwood of each tree and machine stress graded by Forest Research. Paired comparisons of clones and families (stem diameter, stem form and wood density held nearly equal) suggested that structural lumber outturn and $value was higher in clones due to a tighter distribution of stiffness (‘clonal uniformity’) via the absence of low-stiffness stems arising within families by genetic segregation.

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Title : WESTERN HEMLOCK FAMILY TRIALS: INVESTIGATION OF GENETIC SELECTION FOR WOOD AND FIBRE AND IMPLICATIONS FOR FINAL PRODUCT VALUE.

Abstract : Initial investigation of an older western hemlock progeny trial found strong family effects for internal wood and fibre properties including: wood density, average fibre length and fibre coarseness. Heritability estimates for these traits ranged from 0.5 to 0.9. We also found adverse genetic correlations not only for growth rate and wood density but also between growth rate and fibre coarseness. Handsheets and structural wood samples were prepared and sampled on a family basis to test the effect of selection for the wood and fibre traits. We have since extended this work to include many more trial series and more internal wood and fibre characteristics including fibre cell wall dimensions and microfibril angle. We have also tested many more families for their handsheet properties and are extending this to other product values. We are hoping eventually to use this information to define economic weights to use in our breeding program for trait selections. We have also included a silvicultural element to this project by characterizing the wood and fibre values from these trials as inputs into the growth and yield simulator and evaluating these properties in this type of managed forest for potential end-product values. This project has built a substantial data base and a co-operative relationship between forest geneticists, siliviculturalists and wood and paper scientists.

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Title : OPTIMISATION OF MULTIPLE TRAIT SELECTION INCLUDING PULP AND PAPER PROPERTIES AS SELECTION OBJECTIVES

Abstract : Options for incorporating wood quality in British Columbia's hemlock breeding program were investigated. Seventy half-sib families were examined. Attention was given to quantitative variation in tracheid characteristics and its effects on pulp and paper properties. Based on the existing knowledge of relationships between fibre properties and paper quality, the potential gain in yield and wood quality was estimated for different selection strategies. Improvement without much trade-off was possible for volume and tensile strength of pulp and paper. Significant trade- offs would be required to improve the volume, tear strength of paper and strength of mechanical pulp. Therefore, multiobjective optimisation would be beneficial. Conservative selection strategies seem realistic, and compromises with gain in volume growth may be warranted. The potential loss arising from the uncertainty about economic values for objectives can be overcome by using multiple selection indices in multiple breeding populations.

SESSION 5

Chairs, Enrique Ritter / Mitch Sewell

Genetic mapping, QTL detection, candidate genes and proteins, exploitation of macro and micro-synteny

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Title : GENETIC MAPPING IN TROPICAL HYBRID PINES

Abstract : *Pinus elliottii* var *elliottii*, *P. caribaea* var *hondurensis* and its hybrid are the major commercial timber taxa established in plantations in tropical and subtropical Australia. The key commercial traits for timber quantity, quality as well as vegetative propagation traits essential to a clonal forestry program, are the subject of a genetic mapping project. A full-sib hybrid family (2ee1-102 x 1ch1-63) has been identified that is represented in trials and plantations of varying ages and with family block sizes ranging from several hundred to 20,000 individuals. Genetic maps for the two parents have been constructed using AFLP and microsatellite markers. Data on a small sample of genotypes for a range of traits including, branch architecture, wood basic density, bark thickness, percentage of rooted cuttings, early growth and stem form is available and is being assessed for marker-trait associations using several methods to adjust for site and microsite variation. Results and conclusions from these early assessments will be presented.

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Title : THE IDENTIFICATION OF PCR-BASED MARKERS LINKED TO WOOD SPLITTING IN *EUCALYPTUS GRANDIS*

Abstract : WOOD SPLITTING IS A DEFECT IN EUCALYPTUS WHICH RESULTS IN CONSIDERABLE LOSSES WHEN CONVERTING LOGS TO SOLID WOOD PRODUCTS. *EUCALYPTUS GRANDIS* CLONES FROM TWO CLONAL TRIALS WERE SELECTED AND IDENTIFIED AS HIGH AND LOW SPLITTERS. THE SPLITTING VALUES WERE GENERATED BY MEANS OF BACKWARD SELECTION BLP (BEST LINEAR PREDICTION) ANALYSIS USING THE PROGRAMME MATGEN 5.6, A BLP PACKAGE FOR UNBALANCED INDEX SELECTION IN TREE BREEDING. USING THE BULKED SEGREGANT ANALYSIS METHOD FOR RAPID IDENTIFICATION OF MARKERS LINKED TO WOOD SPLITTING, DIFFERENT BULKS WERE GENERATED COVERING SPECIFIC SECTIONS OF THE TAILS OF THE TWO EXTREME GROUPS(SPLITTING VS NON-SPLITTING). THE BULKS WERE SCREENED FOR DIFFERENCES USING AMPLIFIED FRAGMENT LENGTH POLYMORPHIC AND RANDOM AMPLIFIED POLYMORPHIC DNA PRIMERS. TWO PUTATIVE SEQUENCE CHARACTERIZED AMPLIFIED REGIONS (SCARS) GENERATED FROM THE ABOVE METHODS HAVE BEEN LINKED TO SPLITTING FOLLOWING A REGRESSION ANALYSIS.

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Title : CONSTRUCTION AND EXPLOITATION OF A MULTIFUNCTIONAL AND SATURATED GENETIC MAP FOR CONIFEROUS SPECIES

Abstract : The aim of this project is to construct an ultra-high density linkage map (UHD map) of Pinus pinaster based on up to 12.000 AFLP markers and 100 published microsatellites (SSR). The first map has been placed on the Internet (www.neiker.net/UHDfor) and will be exploited to full extent for comparative genome analyses and QTL analysis in different genetic backgrounds. It is the aim to align other published linkage maps in forest species with this reference map. Based on reduced linkage maps derived from a common subset of primer combinations, comparative genome analysis are performed in different pine species and related gymnosperms. QTL analysis for important characters in different forest species will be performed and QTL effects and positions compared. Allele-specific markers for important QTLs will be developed and their variability in local germplasm and breeding material will be analysed. Until now nearly 250 AFLP primer combinations have been evaluated on the P. pinaster reference population (0024 x C803).The observed polymorphisms in terms of segregating fragments was lower than expected due to an increased degree of homozygousity in the parental lines. Sevreal PCs did not give any polymorphic band. Nevertheless, over 1000 AFLP fragments are available for linkage mapping. Two individual parental maps have been constructed consisting each of 12 linkage groups, in agreement with the 12 chromosomes of the pine genome. Total genome length of the parental maps is around 1800 cM. The availibility of anchor points based on fragments common to both parents and based on codominant markers allowed to obtain a consensus map of Pinus pinaster. Numerous SSR and EST markers descending from different pine species have been evaluated in the reference population. Although amplification products were obtained in most cases and polymorphism between parents were observed in many cases, a low degree of segregating polymorphisms was observed also with these marker types. Several SSR and EST markers could be placed on the reference map. Since part of them are also mapped in other populations of different pine species, a partial alignment of our reference population with different other published maps was possible. This project will provide a tremendous amounts of markers which are useful for a broad range of forest species. On one hand they serve for possible isolations of genes in the future. On the other hand it provides markers for important characters which can be applied in early selection tests. This will accelerate breeding of forest species and lead to increased productivity of forests and improved quality of wood products, which in its turn will make forests more sustainable. The UHD map can serve as a reference map for other mapping experiments of forest species in the future.

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Title : CONTRIBUTION OF MARITIME PINE FOR COMPARATIVE MAPPING IN CONIFERS

Abstract : The sequencing of expressed genes (EST) and the development of Simple Sequence Repeats (SSR) provide orthologous markers which are useful for comparative genome mapping in conifers. If the ordering of genes is maintained, then these species can be considered as a single genetic system. At a more practical level, this would allow to transfer information (QTL, candidate genes) between species. This discipline could also allow studying the evolution of their genome. Several research organisations worldwide are developing primer sets to amplify EST and SSR that are used as anchor-points between the genetic maps of approximately ten conifer species belonging to the Pinaceae. In this study about 200 ESTs and 60 microsatellites were tested for amplification in *Pinus pinaster*. Some were mapped and their position was compared to the *Pinus taeda* reference map. It was shown that gene order was well conserved between both species. This report agrees with others pairwise pine genome comparison and shows that pine genomes did not evolve in term of chromosomic rearrangement since their divergence. This encouraging result should allow interspecific validation of QTL location and will ultimately contribute to the development of marker-assisted selection.

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Title : MAPPING QTLS FOR WOOD PROPERTY TRAITS IN LOBLOLLY PINE (*PINUS TAEDA* L.) WITH APPLICATION TOWARDS MARKER-ASSISTED BREEDING.

Abstract : A QTL (quantitative trait locus) analysis is one approach used to understand the genetic architecture of a complex trait. The number of QTLs that segregate within a population, as well as their magnitude of effect, gene action and map position, can be estimated. However, before a commitment is made toward marker-aided breeding (MAB), QTLs must be verified in different genetic and environmental backgrounds. This approach was used in two, related pedigrees of loblolly pine (*Pinus taeda* L.; i.e., *qtl* and *prediction* pedigrees) to detect QTLs associated with physical and chemical wood property traits. Physical traits were assayed using xray densitometry and diffraction and include wood specific gravity (*wsg*), volume percentage of latewood (*vol%*) and microfibril angle (*mfa*). Cell wall chemistry (*cwc*) traits were assayed using pyrolysis molecular beam mass spectrometry (pyMBMS) and include estimates for alpha-cellulose and hemicellulose sugars, and lignin. Approximately 14 unique QTLs for *wsg*, nine for *vol%*, eight for *mfa*, and 10 for *cwc* were detected among both pedigrees. The average phenotypic variance explained by individual QTLs was typically small (~7% and ~11% in *qtl* and *prediction* pedigrees, respectively), as expected for intraspecific pedigrees. Four (29%) QTLs for *wsg*, two (22%) for *vol%*, five (63%) for *mfa*, and four (40%) for *cwc* were verified in both pedigrees. The majority of QTLs exhibited some degree of dominance. These results are currently being used in candidate gene analyses and association tests, and can be used to design MAB strategies for these traits.

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Title : GENETIC DETERMINISM OF WOOD AND END-USES PROPERTIES IN MARITIME PINE

Abstract : The maritime pine breeding programme has now achieved its third generation of selection. Genetic gain of improved varieties reachs 30 % for both volume and straightness. The introduction of wood quality selection criteria is now considered as an important objective in the redesigned programme based on sub-lining strategy. Such selection is however hampered by costly assays, the necessity to wait until the trees are nearly mature to evaluate wood properties and the lack of genetic information for such traits. In order to provide the basic background to initiate a breeding programme for wood and end-uses properties, a three step approach was adopted. In a first step, high throughput methods were optimised to measured wood properties on a large scale. In a second step, genetic parameters were estimated for a wide range of wood quality traits (microdensity, fibre properties, lignin content, pulping related traits). Some of them presented a strong genetic control (e.g. wood heterogeneity, lignin content). Genetic relationships between traits were also obtained and allowed to estimate the response on other traits (e.g. growth). However, despite the identification of possible target traits for breeding, such analysis remains unable to provide (i) early selection criteria and (ii) the basic knowledge of the genetic architecture of these traits (number of genes, location in the genome and effect). Therefore, in a third step, wood quality traits were dissected into their mendelian components (QTLs). Several genomic regions controlling part of the phenotypic variation of wood and end-uses properties were identified, providing a first set of potentially useful tools for early marker-aided selection. The validation of the detected QTLs will be discussed.

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Title : MOLECULAR BREEDING OF CROWN AND BRANCH QUANTITATIVE TRAITS AND THE USE OF CYPRESS (*CUPRESSUS SEMPERVIRENS* L.) AS A MODEL SPECIES

Abstract : The economic importance of quantitative traits associated with crown form and branch habit (crown shape, diameter and angle of lateral branches, etc.) is very significant hence pertinent research is developing. The progress of relevant research that is presented in this communication is based upon: (a) the selection of cypress (Cupressus sempervirens L.) which is found in nature in two forms the horizontal (C. sempervirens var. horizontalis) and the pyramidal (C. sempervirens var. pyramidalis) as a model species, (b) the development of a protocol for rapid and high quality DNA extraction and isolation and the optimization of the application of PCR/RAPD markers, (c) the investigation on the levels of molecular genetic variation in cypress natural populations and (d) in the employment of bulk segregant analysis in a particular genetic background on the aim of mapping crown and branch traits. The detection of notable molecular variation in cypress natural populations can be viewed as a prerequisite for future intensive breeding activities. Particularly the discovery of RAPD genetic markers closely linked to a genomic region associated with crown form in cypress, which was achieved, is regarded as a result of wider importance in light of synteny and linkage conservation results above the species level that comparative genomic mapping studies have produced in recent years. Pertinent discussion also addresses the questions of the detection of genetic markers even more closely associated with the genomic region in question, which may proceed in the future, even in cloning of such gene(s). A strategy for the application of molecular breeding in perennial woody plants is also introduced.

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Title : GENETIC CONTROL OF MICRODENSITOMETRIC COMPONENTS OF WOOD CHARACTERISTICS IN HYBRID LARCHES

Abstract : The use of wood quality traits as selection criteria in forest breeding is hampered by long delays before evaluation. The feasibility of early selection using genetic markers cosegregating with the trait of interest, or inolved in its control, could change this situation. Wood quality traits have been for a long time restricted to wood density assessed with a pilodyn in order to avoid tree destruction. Microdensitometry have already proved its capacity to provide several parameters on wood quality traits such as early or final wood density, wood density heterogeneity and radial growth without tree destruction. Moreover microdensitometry can provide parameters for each ring which allows detection of genetic expression variation among years. Analyses have been carried out on 16-year-old trees from a 12 x 12 factorial mating design between European and Japanese larches. A genetic map based on AFLP, RAPD and ISSR markers has been used for QTL detection. Several genomic regions are involved in the control of wood de nsity components.

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Title : IDENTIFICATION OF MOLECULAR MARKERS FOR SELECTED WOOD PROPERTIES OF NORWAY SPRUCE (*PICEA ABIES* (L.)KARST).

Abstract : Norway spruce (Picea abies (L.) Karst) is one of the most important forest trees of the northern hemi-sphere. Among the different wood properties like chemical characteristics (lignin content and extractive content), fibre characteristics, resistance etc., wood density is a key property highly correlated with lumber as well as pulp yield. A high and uniform wood density is desireable for many solid wood products, while high or low wood density is favourable depending on the paper product and process considered. Wood density is mainly influenced by fibre geometry and fibre wall thickness. The fibre formation is controlled by physisological processes depending on genetic and enviromental conditions along cambial ageing. The aim of the European-project "GENIALITY" supported by the European Commision is the genetic improvement of wood quality and the increase of selection efficiency for different end uses. Within this project an essential part is the identification of molecular markers for selected wood properties of Picea abies, which can be used in early selection tests. The approach for the identification of molecular markers especially for wood density and extractives content was followed the strategy of bulked segregant analysis (BSA). Different pools were con-structed from the most extreme individuals for high and low wood density /extractives content. Identi-fication of molecular markers was carried out by using a nonradioactive AFLP-analysis (ALF-express). For wood density 102 AFLP-Primer combinations (Mse-Pst; Mse-Eco;Mse-Hind) were tested in total and 107 polymorphic AFLP-fragments with potential linkage to high and low wood density respectively were identified. 23 polymorphic fragments were selected for further analysis and tested by contemplation of marker presence/absence for individuals in each pool and validated in different populations segregating for high and low wood density. In total four fragments for wood density and one fragment for low extractives content were identified.

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Title : GENETIC CONTROL OF QUANTITATIVE TRAIT VARIATION IN POPULATION OF *PINUS SYLVESTRIS L.*

Abstract : Pinus sylvestris demonstrates large genetic variation for many quantitative traits such as frost hardiness, growth rhythm and terpene composition within populations in Sweden. The traits are also clinally related to the latitude of origin as a result of climatic adaptation after the last glaciation. For several terpene components, simple mendelian inheritance has been found. We have used DNA markers to map QTL controlling the pattern of variation in frost hardiness and growth rhythm in half-sib and full-sib families. We found several QTLs explaining different proportions of the phenotypic and additive genetic variances for frost hardiness in spring and autumn and various growth traits. Some QTLs appeared to have pleiotropic effects on several traits. The patterns of variation in frost hardiness and growth rhythm are controlled by the accumulative action of several QTL alleles. Clinal variation in autumn frost hardiness is a result of the accumulative action of both major and minor QTL allelic differences between populations. In northern populations, with better autumn frost hardiness due to an early cold acclimation rhythm, major QTL alleles with strong effects on hardiness may be more frequent than in southern populations. In this paper we present some views upon how allelic frequencies for different QTLs affecting frost hardiness may vary among populations of Pinus sylvestris. _

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Title: CASCADE: SECURING GENE CONSERVATION, ADAPTIVE, BREEDING POTENTIAL AND UTILIZATION OF A MODEL MULTIPURPOSE TREE SPECIES (*CASTANEA SATIVA* MILL.) IN A DYNAMIC ENVIRONMENT

Problems to be solved
The CASCADE project seeks to provide the information needed to safeguard European chestnut, an important tree for landscape and rural diversification which has suffered from domestication, fungal attack and silvicultural practices - challenging its genetic variation - and whose genetic resources are at risk from expected climatic change for future generations. Three main problems will be addressed: (1) to assess the capacity of chestnut stands (both natural and domesticated) to cope with future climate changes, biological (pathogens) and anthropic (silvicultural practices) threats; (2) to devise optimized long-term strategies for conservation of evolutionary and economic potential of chestnut stands in rural areas; (3) to provide operational, effective criteria, methods, guidelines and recommedations helping in the optimal management of extant and future chestnut cultivation. The objectives of CASCADE are also consistent to some of the aims of EU policies (EC Agenda 2000, European Council Resolution 1999/C56/01, Regulation EC No 1467/94, etc.) in the fields of biodiversity conservation, sustainable forestry, environmental protection, rural development.

Scientific objective and approach
CASCADE aims to characterise the variability of wild and cultivated chestnut stands from across Europe, with respect to its genetical, ecological, pathological, economical and silvicultural aspects. A range-scale inventory of chestnut genetic resources will be prepared, integrating molecular, physiological and pathological data, with historical and climatic information. DNA- and protein-based variation will be used for estimation of genetic diversity of stands across the present distribution area. Genetically depauperated populations will and will be highlighted as well as stands threatened by pathogen attack. Mechanisms of maintenance of genetic variation (e.g., gene flow via pollen dispersal) will be elucidated. The genetic basis of variation for adaptive traits (drought resistance, phenology and growth, resistance to pathogens) will be investigated on seedlings reared in greenhouses and in field trials. Assessment and comparison of the adaptive potential of different stands to contrasting environmental conditions will help in the identification of genetic "reservoirs" of adaptability to the climate change. Conservation and sustainability indexes will be developed taking into account the relations between species' autoecology, management types and economic values from cultivation. Cost-benefit analysis of specimen programs for conservation of chestnut genetic resources will be carried out on market and non-market benefit and costs as well as by a survey-based approach.

Expected Impacts
The large inventory of chestnut resources will be useful for the establishment of conservation priorities by the delineation of both endangered and 'functional gene resource' populations. Estimation of pollen dispersal in orchards will help to define technical guidelines for sustainable local silvicultural practices (min/max number and nature of the pollinators, min/max distances of abandoned orchards to be recovered, etc.). The identification of genetically valuable material (high-performing and/or high-resistant chestnut individuals useful as starting material for the genetic improvement) will be obtained as a spin-off of the adaptive traits analysis. Genetic information needed to inform future management policy will be provided as well as socio-economic evaluation of the costs and benefits of different conservation approaches. Criteria for the future sustainable management of chestnut genetic resources will be established and promulgated to conservation bodies, ministries and farmers so to safeguard its future. CASCADE will also provide a model for similar studies on other multipurpose tree species.

SESSION 6

Chairs, Pramod Gupta / Sara von Arnold

Somatic embryogenesis and tissue culture

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Title : AUTOMATED SELECTION OF GERMINATION-COMPETENT CONIFER SOMATIC EMBRYOS BY MULTI-VIEWPOINT COLOR IMAGE ANALYSIS

Abstract : Selection of the germination-competent fraction of somatic embryos produced in petri-plates or bioreactors is an important step in the production of manufactured seed and clonal planting stock. At present, it is a costly and rate-limiting step, carried out manually by trained technicians using imprecise morphological criteria. Our goal is to eliminate it as a significant cost component by using automated selection. To this end, somatic embryos of several Douglas-fir genotypes were aseptically color-imaged from three viewpoints perpendicular to one another; classified as morphologically acceptable or not, and tested for germination. Images were pre-processed, and analyzed in a way that emphasized the use of all pixel information (not just perimeter pixels) in the construction of classification models. Image principal components ("eigenembryos") and over 700 metrics, computed from each view in "training sets" of 600 or more embryos, were used to develop classification models for each viewpoint. These intermediate classifications were combined using Bayes Optimal Classifier to produce more robust, combined models, and the models evaluated for accuracy and stability based on the results of 100 simulations that started with random selection of new training and test sets. The most accurate and stable models thus selected were capable of classifying for acceptable morphology with an accuracy (defined as the average of alpha and ß, the fraction of embryos correctly classified as good and poor respectively) ranging from 80-90% over 4 genotypes. This accuracy was related to an increased germination in the selected population, G_s, by Ibaraki's formula: $G_s = G_o / (G_o + ð(1-G_o))$, where G_o is the germination in the original population, and $ð = (1-ß) / alpha$. For a morphology-trained classification model, G_s was about 7 percentage points lower than G_s achieved through manual selection. However, for a model trained directly against germination truth data, G_s was only 1.5 percentage points below that achieved through manual selection. For both morphology- and germination-trained models, the rejection rate of good embryos (1-alpha), an economically important measure of wastage during the selection process, was only three percentage points higher than corresponding manual error, and classification could proceed at a potentially much faster rate than by hand. The models produced by this software sequence contained metrics and principal components that were specific to the genotypes in question. Hence the process can be applied to lines of differing morphology and competence, without the need for expert selection of morphological input variables.

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Title : SOMATIC EMBRYOGENESIS IN NORWAY SPRUCE, WITH SPECIAL EMPHASIS ON EARLY EMBRYO DIFFERENTIATION

Abstract : Growth of somatic embryo plants is under a cumulative influence of the treatments given during proliferation, maturation, desiccation and germination. Therefore, in order to develop a safe method to produce somatic embryo plants in a large scale, it is important to know how the processes are regulated. The developmental pathway of somatic embryos of Norway spruce involves two distict phases. The first phase is represented by proliferation of proembryogenic masses (PEMs) and the second phase encompasses development of somatic embryos. The transition from PEMs to somatic embryos is a critical step, which is triggered by withdrawal of plant growth regulators.Formation of somatic embryos is accompained by massive cell death. The importance of PEM-to-embryo transition will be discussed.

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Title : SOMATIC EMBRYOGENESIS IN SILVER FIR (ABIES ALBA MILL.)

Abstract : At the northern border of its natural distribution, e.g. in the mountains of Saxony, A. alba is a rare and endangered species. The genetic diversity is low, and natural regeneration is insufficient. The aim of this project was to use somatic embryogenesis (SE) for multiplication of valuable seed lots produced by controlled pollination. In the first step, methodical studies were undertaken to evaluate the selectivity of the induction and propagation process, i.e. the percentage of the initial number of genotypes which finally could be propagated by SE. Experimental details of SE in A. alba were similar to those described for A. nordmanniana (Rahmat et al., in this volume). Seed lots from four locations in Saxony were compared, with more than 700 seeds in total. In 52% of the zygotic embryos SE was observed. Seventy-six percent of the embryogenic clones could be established and propagated. The genetic characterisation (isozyme patterns) of the established clones indicated a selection of genotypes with higher levels of heterozygocity. In 85% of the established clones morphologically normal somatic embryos matured. The embryo yield showed a considerable variation. In 73% of the established clones greater than 25 mature embryos per g FW of embryogenic tissue were obtained, and in 31% of the clones between 100 and 530 mature embryos per g FW were produced. Conversion, soil establishment and cryopreservation are in progress.

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Title : EFFECT OF AUXIN INHIBITION ON MATURATION OF EMBRYOGENIC TISSUE CULTURES OF NORDMANNS FIR (ABIES NORDMANNIANA)

Abstract : In general, the growth regulator auxin is essential for continued proliferation and growth of embryogenic tissue cultures of conifers. The transition from proliferation to maturation is normally controlled by a shift in the composition of growth regulators, were auxin is excluded and ABA is included in the growth medium. However, for Nordmanns fir and other Abies species auxin is not needed for proliferation and in many cases inclusion of ABA does not stimulate the transistion from proliferation to maturation. Maturation of Nordmanns fir has been hampered by continued proliferation of the culture during maturation, and that has until now been the major problem in development of large scale propagation methods for this species. Among a number of tested anti-auxins, the auxin antagonist PCIB showed a remarcable effect. In all tested genotypes, proliferation stopped and maturation was initiated when the cultures were transferred to maturation medium including ABA and PCIB. The optimum application period and the concentration of PCIB, was genotype dependent, but for over 100 tested genotypes maturation was strongly increased by application of 2 standard protocols. At present, the developed methods are tested in large scale for establishment of field trials.

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Title: APPLICATION OF SOMATIC EMBRYOGENESIS FOR MICROPROPAGATION OF *ABIES ALBA* MILL. POLISH, NATIVE ORIGIN.

Abstract: Somatic embryogenesis of *Abies alba* Mill. was achieved following to the method of Hristoforoglu et al. (1995) with some modifications. Mature embryos were cultured on an MCM basal medium (Bornman and Jansson 1981) and on GD medium (Gupta and Durzan 1986). Both media were supplemented with 2.2 µM BAP, 2.3 µM kinetin and additionally the MCM medium was used with 4.4 µM BAP. Induction frequencies of ESM (embryogenic suspensor mass), using mature, zygotic embryos were 1 %-29.4 %. The immature embryos were placed on SH medium (Schenck and Hildebrandt 1972) with 4.4 µM BAP or on MCM medium with 2.2 µM BAP and 2.3 µM kinetin. ESM induction frequencies using immature embryos were 0-10.4 %. For ESM proliferation the MCM basal medium was supplemented with 2.2 µM BAP, 2.3 µM 2,4-D with different concentrations of casein hydrolysate (0-1500 mg/l). The calli grow significantly ($p<0.05$) better on medium supplemented with 1500 mg/l of casein hydrolysate. Seasonal changes in ESM fresh weight increment have been observed during the year. For maturation pieces of ESM were placed on the basal medium with different concentrations 20 - 80 µM of ± cis-trans ABA - with or without 1 µM IBA and PEG (3-7.5 %, 3350). The addition of 1µM IBA and 3% PEG into the maturation medium with ABA improved embryo development. Mature somatic embryos needed to be partially dried at relative high humidity for 2-3 weeks and then placed on the germination medium. Plantlets were planted to Jiffy pots or to a mixture of peat and vermiculite. The studies are in progress.

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Abstract: Somatic embryogenesis has a potential to be used for fast clonal propagation of superior genotypes and for production of large number of trees in a short time. The initiation, development and conversion of somatic embryos to plants in Picea abies and Abies alba was studied. Factors studied included the effects of phytohormones, osmotica, duration of embryo drying and photoperiod. Embryogenic tissue was maintained in a juvenile state by cryopreservation in liquid nitrogen and genetic stability was investigated. Different genotypes were used for the establishment of embryogenic cultures. The high frequency of somatic embryo initiation was achieved in Picea abies (56 - 92 %) and Abies alba (23 - 58 %) provenances growing in Czech Republic, using immature and mature zygotic embryos as initial explants. The use of ABA together with non-permeating osmoticum (PEG 4000) led to the improvement of somatic embryo and to higher yield of mature somatic embryos containing normal storage proteins, and increased the ability of embryos to survive the drying treatment. Slow drying of somatic embryos increased germination frequency and improved root and shoot elongation. Conversion of mature somatic embryos to plantlets ranged from 43 to 92 % (Picea abies) or 22 to 64 % (Abies alba) depending on the genotype.Produced micropropagated somatic trees of Picea abies and Abies alba were planted in he field and their growth performance was observed for 10 - 15 years. High field survival of somatic trees was achieved. After 10 - 15 years of tree growth in forest experimental plots, the height and diameter dimensions of somatic trees were comparable to those of trees produced from seeds. Obtained results indicate that in vitro regenerated trees were genetically uniform in comparison with the original genotype. Growth of trees regenerated from somatic embryos was orthotropic and morphological abnormalities in the shape and form of needles, branches and stem were not observed.

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Title : PLANTLET FORMATION FROM SOMATIC EMBRYOS, AND CRYOPRESERVATION OF EMBRYOGENIC CELL LINES OF *PINUS ROXBURGHII* SARG.

Abstract : AMONG THE NON-CONVENTIONAL METHODS OF PROPAGATION, SOMATIC EMBRYOGENESIS IS PRESENTLY BEING SUCCESSFULLY EMPLOYED FOR THE LARGE-SCALE PROPAGATION OF PINES. *PINUS ROXBURGHII* SARG. OR CHIR PINE, IS AN INDIGENOUS AND IMPORTANT PINE SPECIES OCCURRING IN THE LOWER ALTITUDES OF THE WESTERN HIMALAYAS. CONSIDERING THE NEED FOR THE PROPAGATION OF ITS ELITE VARIETIES, WE HAD STARTED THESE STUDIES DURING THE YEAR 1995. SINCE THEN, WE HAVE BEEN ABLE TO ESTABLISH THE EMBYROGENIC CELL LINES OF CHIR PINE AND ALSO ACHIEVED PLANTLET REGENERATION. THIS PIECE OF WORK HIGHLIGHTS THE STUDIES UNDERTAKEN FOR THE CONVERSION OF SOMATIC EMBRYOS AND ALSO THE METHODS USED FOR CRYOPRESERVATION OF THE EMBRYOGENIC CELL LINES. IT ALSO DISCUSSES THE IMPEDIMENTS THAT WE FACED IN CONVERSION OF SOMATIC EMBRYOS FROM 3-4 YEAR OLD EMBRYOGENIC CELL LINES. INITIAL STUDIES INCLUDED THE SCREENING FOR THE CORRECT STAGE OF IMMATURE ZYGOTIC EMBRYO OR THE `WINDOW' AND INITIATION OF SOMATIC EMBRYOGENESIS BY EXTRUSION FROM THE MEGAGAMETOPHYTES. A NUMBER OF EXTRUSIONS THAT ESTABLISHED AND PROLIFERATED WERE MAINTAINED AS DIFFERENT CELL LINES. A PRE-TREATMENT ON HORMONE FREE MEDIA CONTAINING ACTIVATED CHARCOAL FOR 3-4 WEEKS WAS ESSENTIAL FOR THE PRIMING OF THE EMBRYOGENIC TISSUE FOR MATURATION TREATMENT. MATURATION TREATMENTS INCLUDED CULTURING ON VARIOUS CONCENTRATIONS AND COMBINATIONS OF ABA, PEG, MALTOSE AND GELLAN GUM (MATHUR ET AL., 2000). THE NEXT IMPORTANT STEP FOR SUCCESSFUL CONVERSION OF THE SOMATIC EMBRYOS IS DESICCATION TREATMENT. NON-DESICCATED SOMATIC EMBRYOS WERE OBSERVED TO GERMINATE PRECOCIOUSLY WITHOUT FURTHER GROWTH OR SHOOT ELONGATION. THE CELL LINES FROM 1996-97 STUDIES THAT HAVE PASSED THROUGH MORE THAN 30 SUBCULTURES, WERE EARLIER FOUND NON-RESPONSIVE TO MATURATION TREATMENTS. ALTHOUGH, AFTER WASHING THIS TISSUE AND CULTURING FOR 6-8 WEEKS ON HORMONE FREE MEDIA PRIMED THE TISSUE FOR MATURATION, HOWEVER THE SOMATIC EMBRYOS PRODUCED WERE ABNORMAL. THIS CLEARLY HIGHLIGHTS THE IMPORTANCE OF CRYOPRESERVATION OF FRESHLY ESTABLISHED EMBRYOGENIC CULTURES. WE HAVE BEEN SUCCESSFUL IN REGROWTH OF THE CRYO-STORED (IN LIQUID NITROGEN) EMBRYOGENIC CULTURES AFTER THEIR PRECULTURE WITH CRYOPROTECTANTS (DMSO AND SORBITOL), SLOW FREEZING AND FAST THAWING. THE VIABILITY OF THE REJUVENATED TISSUE WAS CHECKED BY FDA STAINING AND THE GENETIC FIDELITY WAS CONFIRMED WITH ISSR PRIMERS. THE EXPERIMENTAL DETAILS OF THIS WORK WILL BE DISCUSSED IN THE PRESENTATION. **REFERENCE:** MATHUR G., VON ARNOLD S. AND NADGAUDA R.S. (2000) STUDIES ON SOMATIC EMBRYOGENESIS FROM IMMATURE ZYGOTIC EMBRYOS OF CHIR PINE (*PINUS ROXBURGHII* SARG.) CURR. SCI. 79(7): 999-1004.

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Title : CHILLING OF IMMATURE EMBRYOS, SUSPENSION CULTURES AND MATURATION OF SOMATIC EMBRYOS OF SCOTS PINE (*PINUS SYLVESTRIS* L.)

Abstract : Conifer somatic embryogenesis can be used as a tool for breeding programs; in adittion this technique can be utilized for genetic transformation assuring the regeneration of whole transformed plant. Biobalistic protocols can be used, however the previous requirement is the regeneration of somatic embryos from suspension cultures. Our aim was to achieve mature somatic embryos from selected genotypes that were treated by chilling and later regenerated from suspension cultures. The best genotypes, which produce high quality somatic embryos in solid medium, were selected. Following the protocol developed for *Pinus maximartinezii* (Ramirez-Serrano 1996) were applied the chilling treatment from 1 to 11 months and subsequently suspension cultures. After that, the immature embryos from suspension cultures were treated on growth regulators lacking media supplemented with activated charcoal in order to improve the response to maturation promoter. Maturation media where supplemented with low ammonium to nitrate molar ratio, abscisic acid and gellan gum. High quality and yield of mature somatic embryos were achieved. In order to avoid precocious germination the cotyledonary somatic embryos were partly desiccated under high relative humidity. The results shown chilling treatment and suspension cultures assure mature somatic embryos of Scots pine (*Pinus sylvestris* L.).
Acknowledge to CONACyT Mexico for the giving support and payment of the cost related with my studies at the Swedish University of Agricultural Sciences. Ramirez-Serrano, C. 1996. Embryogenesis somática en Pinus maximartinezii Rzedowski. MS Thesis. Universidad de Guadalajara. México. Pp. 80.

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Title : INITIATION OF SOMATIC EMBRYOGENESIS FROM CONTROLLED CROSSES IN PINUS PINASTER AIT.

Abstract : Pinus pinaster is one of the most important species for the French wood and paper industries. It covers more than 1 million ha. Strong efforts are focused to genetically improve the species and make available more efficient sylviculture practices. Somatic embryogenesis is presently considered as part of the breeding programs. This paper presents a summary of AFOCEL results on initiation of somatic embryos clones from immature seeds since the early experiments in 1988 until 2000 with a focus on unpublished data (year after 1994). Immature embryos from controlled pollinated elite trees have been harvested during the summer time, taking into account the competence window defined in late 80's. The initiation rate of embryonal suspensor masses (ESM) was strictly scored at the level of stabilized proliferating material to avoid artificial overestimation. It was revealed to be highly variable both among crosses and among years for the same cross despite harvesting of plant material at the same time of the year and using only embryo at the precise developmental stage defined as optimal. Medium comparison during rapidly concluded to the superiority of DCR, which allows more regular and higher results on average despite not being the best in each particular case. In addition to precluding the checking of the developmental stage of each zygotic embryo (ZE) put in culture, presence of the megagametophyte tends to give us a lower initiation rate. No correlation between ZE development and temperature data could be observed, especially during the last years that were significantly warmer in the Landes area where are located the seed orchards. No strict parental effect could be seen but availability of cones of a particular cross is difficult to obtain regularly each year, making very hazardous the possibility to replicate experiments. The results merely suggested a physiological influence of the mother tree rather than a strictly mono- or bi-parental genetic effect. When weather and cone characteristics are good, initiation rates average 45%, reaching 75% for some crosses with the recovery of ESM from each family. Key words: Pinus pinaster, initiation, genetic improvement

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Title : SOMATIC EMBRYOGENESIS IN LOBLOLLY PINE (PINUS TAEDA L.): IMPROVING CULTURE INITIATION RATES

Abstract : Loblolly pine is one of the most important commercial trees in the U.S. To be successful for commercial use, Somatic Embryogenesis Technology must work with a variety of genetically diverse seeds. Initiation rates of loblolly pine (Pinus taeda L.) were improved through a combination of modified ½ P6 Salts, activated carbon at 50-100 mg/l, copper and zinc added to compensate for adsorption by AC, 1.5% maltose, 2% myo-inositol, (to raise osmotic level partially simulating the ovule environment), 500 mg/l case amino acids, 450 mg/l glutamine, 2 mg/l NAA, 0.45 mg/l BAP, 0.43 mg/l kinetin, and 1.6-2 g/l Gelrite. Across 10 open-pollinated families initiation rates ranged from 3-33% averaging 16%. When 98 resulting cultures were tracked over a six-month period of continuous 2-week subcultures on semisolid medium only 20% survived. The key features resulting in the development of this medium will be discussed.

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Title : ADVANCEMENTS IN SOMATIC EMBRYOGENESIS OF PINUS STROBUS AND THE IMPLICATION FOR OTHER PINE SPECIES.

Abstract : Recently a significant progress has been made in somatic embryogenesis (SE) of Pinus strobus both in initiation and maturation stages (Klimaszewska et al. 2001). This progress was achieved mainly by manipulation of the plant growth regulators (PGRs) in the culture medium. PGR at a lower than "standard" concentration significantly increased the SE initiation response and also had a long-term positive effect on the number of mature somatic embryos. Other factors that were tested on maturation of somatic embryos included amino acids, sugars, culture technique and gellan gum concentration. Gellan gum concentration and culture technique that determined water availability during the somatic embryo maturation phase had the strongest impact whereas sugar concentration played a complementary role. An effort to define a maturity marker for somatic embryos will be discussed. The improvements made in SE protocols of P. strobus were tested with other pine species such as P. monticola and P. banksiana. The two latter pines are relatively recalcitrant particularly in the SE initiation and maintenance phase. Some results on methods for circumventing these problems will be presented.

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Title : USE OF TISSUE CULTURE FOR CLONAL PROPAGATION AND LONG-TERM STORAGE OF VALUABLE GENE POOL OF FOREST WOODY PLANTS

Abstract The results of long-term researches on use of tissue culture method for preservation and reproduction of the representatives of valuable gene pool of forest woody plants have been presented. There have been worked out technological methods of clonal micropropagation in vitro of mature (20-33 years old) trees economically valuable birch, aspen and poplar varieties. - Long term (during 9 years) investigations show the possibility of use the technology in vitro for creation of Karelian birch (Betula pendula Roth. var. carelica Merkl.) plantations with earlier display of external characters of patterned wood (from the age of 3-4, and their 100% display by the age of 5-8). Plants, which grown by routine methods (seed method), the characters of patterned texture are displayed later – at the age of 10-12. - There was revealed a valuable for the practical relations peculiarity of micropropagated clones of triploid varieties of white (Populus alba L.) and gray (P. canescens Sm.) poplar whose propagation is difficult to perform by routine method (cutting in a greenhouse – in vivo). It consists in that the productivity of parent plantation (ability of shoot formation, the number of cuttings from one bush and their rootage ability in a nursery), established from propagated in vitro clones, was on the average 1,5 as high as on the plantation established by routine method. It may be connected with higher level of rejuvenilisation of plants propagated in vitro. Similar investigations are carried out with birch and aspen, which are characterized by weak ability to vegetative propagation. Plantations cultures of 5 economically valuable Karelian birch forms (now they are 9 years old), parent plantation of 5 triploid varieties of white and gray poplar are established of micropropagated clones. - There were shown 2 methods of long term in vitro storage of the collection of birch, poplar and aspen valuable genotypes. 1. Long-term (over 8-10 years), multiple and regular (once every 2-3 month), year-round shoot multiplication of birch and poplar regenerants on hormone- free nutrient media without visible sings of ontogenetic aging and somaclonal variation in clones formed, and without the loss of the ability of microcuttings to root and grow normally. This method may be used both for storage and for annual mass clonal propagation of mature trees of the same species. 2. The storage of the poplar and aspen collection in darkness, at 8-100C on hormone-free nutrient medium, supplemented with 20 g/l sucrose, allows for prolongation of subculture period up to one year, preserving regeneration abilities of cultures. Coefficient of propagation was not changed. After 24 months of the storage 90-100% of cultures survived, but in the conditions of routine cultivation (260C at an illuminance 1-2 klx for 16 h per day) in 7 months survivability decreased up to 0-20%. Long term cultivation solves the problem of long-term preservation of valuable forest gene pool ex situ.

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Title : STUDIES ON THE ROLE OF ENOGENOUS AUXIN IN SOMATIC EMBRYO DEVELOPMENT OF EUROPEAN LARCH (LARIX DECIDUA MILL.)

Abstract : In conifer somatic embryos the shift from axial to lateral symmetry occurs during the transition from stage II (globular shape) to stage III (def. of stages see HAKMAN and v. ARNOLD, Physiol. Plant.72: 579-587, 1988). Embryo maturation in our model species occured on auxin-free culture medium. This provides an opportunity to study the role of endogenous auxin in establishing embryo symmetry by using auxin transport inhibitors. The polar transport inhibitor NPA (naphthyl phtalamic acid) had a dramatic influence on formation of polarity and symmetry but did not reduce the number of embryos that matured. Concentrations from 10 to 50 µM were applied to the culture medium and were present during the 6-week period of maturation. Concentrations of 10 and 20 µM NPA disturbed symmetry formation in nearly all embryos. Fusion of cotyledons resulted in a trumpet-like morphology. The embryos were clearly polar, and all embryo organs were developed. However, the apical meristems in most of the embryos had lost their function. A concentration of 50 µM inhibited polarity formation, histogenesis, symmetry formation and meristem function completely. The embryos increased in size, but remained in the globular stage. The results correlated with those of similar studies in excised zygotic embryos of angiosperm species and underline the important role of auxin as an intercellular signal in embryo development. Polar auxin transport might be a prerequisite for the manifestation of polarity and the formation of lateral symmetry as well as for the development of functional apical meristems.

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Title: **CHANGES IN PLASMA MEMBRANE PROPERTIES DURING NATURAL AND ACCELERATED AGEING PROCESSES IN NORWAY SPRUCE (*PICEA ABIES* L. KARST) SEEDS.**

Abstract: The present study examines the mechanism of natural and accelerated ageing process in Norway spruce (*Picea abies* L. Karst.) seeds stored at low temperature for one (control) and eight years. The analyses of vitality, electrolyte leakage, protein composition and activity of the proton-pump ATPase (P-ATPase, EC.3.6.1.35) in plasma membrane of Norway spruce seeds were carried out. One year old seeds from northeastern Poland have been subjected to the accelerated ageing treatment and compared to 8 year-old seeds taken from the same provenance. The vigor and the vitality of the Norway spruce seeds depend on the storage period. After 6 days of experiment both groups of seeds (with accelerated ageing treatment and the 8-year-old) revealed almost the same level of germination and similar electrolyte leakage. Analysis of protein composition in the extracts showed an increased amount of some low molecular-weight proteins (less than 40 kD) in accelerated and naturally aged seeds compared to the control. Our results indicate similar level of P-ATPase activity in natural, accelerated aged and control seeds. This suggests that natural ageing process can involve changes in plasma membrane protein composition but does not affect the P-ATPase activity.

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Title: SOMATIC EMBRYOGENESIS IN EUROPEAN PINES.

Abstract: In Europe, Scots pine (Pinus sylvestris L.) and Maritime pine (Pinus pinaster Ait.) are two major species of great economic interest. Scots pine is essentially a pine of Northern Eurasia whereas Maritime pine is a pine of the Mediterranean region. Somatic embryogenesis was initiated for both species which displayed different propensities. Modified Litvay's medium, with or without growth regulators, was chosen. In P. sylvestris, the highest initation frequency was obtained with intact megagametophytes containing embryos at the four-cell stage to the stage of cleavage polyembryony on both media. In P. pinaster, the best response occurred from excised zygotic embryos at the stage prior to elongation of cotyledon primordia and on medium with growth regulators. Another characteristic distinguishing the two species in culture was that in some embryogenic cell lines of P. sylvestris, somatic embryos matured spontaneously when initiated and maintained on medium without growth regulators. Some of these embryos developed into plantlets on the same medium. Therefore, in P. sylvestris all the stages of somatic embryogenesis were achieved on the medium without growth regulators. However, in both species, maturation of a large number of somatic embryos was greatly improved on medium containing high concentration of gellan gum (Gelrite 10 gl-1) and abscisic acid. Cotyledonary somatic embryos subsequently germinated and developed into plantlets. Germination and plantlet frequencies were higher for P. sylvestris than for P. pinaster. Clonal propagation by somatic embryogenesis is an efficient tool for precise estimation of genetic value and for mass production of genetically improved material. Genetic engineering, which relies on tissue culture systems for gene transfer, offers a means for the introduction of novel traits into important tree species. For maritime pine, transformation of embryonal masses via A. tumefaciens has been realised with our improved protocol developed in hybrid larch. Compared with this one, preliminary results with P. pinaster showed low transformation rates. Transformation protocol will be optimised in order to apply this technique to introduce genes of interest, especially genes related to wood quality in the frame of the european GEMINI project.

SESSION 7

Chairs, Francisco Canovas / Mike Becwar

Molecular biology and genetic engineering

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Title : STABLE STAGE-SPECIFIC EXPRESSION OF THE GUS-GENE UNDER CONTROL OF THE MAS-PROMOTER IN DEVELOPING SOMATIC EMBRYOS OF LARIX DECIDUA MILL. AFTER AGROBACTERIUM - MEDIATED TRANSFORMATION

Abstract : Based on published results concerning Agrobacterium-mediated transformation of Larix sp. (e.g. LEVEE et al., Plant Cell Rep 16: 680-685, 1997), we optimised the procedure for Larix decidua using A. tumefaciens strain GV 3101. This strain harboured the plasmid pPCV 812 and carried the reporter gene gus under control of the MAS-Promoter and the selectable marker hpt under control of the NOS-promoter. As target tissue, embryogenic cultures (ESM) from solid medium as well as suspensions were used. Co-cultivation on of transformants MSG medium occurred for 2 days, and Agrobacterium were eliminated by subculturing on medium containing 500 mg/l cefotaxime. Transformed cells were selected on MSG medium containing 5 or 7.5 mg/l hygromycin. Hygromycin was highly selective in L. decidua. In nearly all resistant lines, the presence of the transgenes could be confirmed by PCR. The procedure was very efficient and yielded on average 3 to 10 transformants per g FW of ESM. The expression level of the reporter gene varied depending on the target genotype, the line and the developmental stage of the somatic embryos. Proliferating ESM did not or only rarely express GUS. GUS-expression increased during embryo maturation up to 28 % of the embryos in stage IV (fully mature embryos). In the first days of conversion, 75 % of the plantlets showed a clear expression. In most of the cases, the expression was located in the base of the hypocotyl and the root primordium, and with lower intensity in the base of the cotyledons and the plumula primordium.

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Title : RAPID PRODUCTION OF FERTILE TRANSGENIC SWEETGUM (*LIQUIDAMBAR STYRACIFLUA* L.) TREES

Abstract : Time is a major constraint in the progress of tree improvement program. Normally forest trees take a decade or two to reach sexual maturity and flower, which renders traditional tree breeding ineffective for germplasm improvement. Biotechnologies have the potential to enhance tree improvement programs. For example, there is the potential for creating new types of trees by introducing novel traits in a relatively short time. In this paper, we report that an effective protocol has been developed for rapid production of fertile transgenic sweetgum trees. A total of 26 transgenic plants of sweetgum were obtained from three independent experiments using the Agrobacterium-mediated transformation system. Integrative transformation and expression of the introduced foreign genes (NPT II and GUS genes) were confirmed using standard assays. The resulted transgenic trees have been maintained in a research greenhouse where they started flowering within five years. Those flowering fertile plants also showed the expression of the marker genes. Thus, the method developed in our lab achieved rapid production of fertile, transgenic trees that are very valuable to the studies of both genetic inheritance and expression of the introduced genes in transgenic trees. Although fertile transgenic plants are routinely produced in many agronomic crops as well as annual species, production of fertile transgenic trees has not previously been reported.

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Title : DIFFERENTIATION OF XYLEM CELLS IN 35S-*ROLC* TRANSGENIC ASPEN TREES

Abstract : In order to investigate the impacts of the *rolC* gene on wood-biological traits, wood structure and the differentiation of xylem cells of 35S-*rolC* transgenic hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.) were analysed and compared with non-transformed control trees. The transgenics are characterized by dwarfism, altered physiological parameters, light green leaves of reduced size, and a precociuos bud as well as leaf development. Histometric measurements revealed thinner fibre walls as compared to the controls. PATAg and potassium permanganate staining in electron microscopy as well as UV-microspectrophotometry of individual cell wall layers did not reveal distinctive differences in the architecture and chemical composition of xylem cells between transgenics and controls, but there was a difference in the dynamics of xylem cell differentiation. In the transgenics the formation of xylem cells was delayed and the differentiation zone reduced to a few rows only. Therefore secondary wall formation was found in very early developmental stages - even in cells which were just deviding in that very moment. Immunocytochemical analyses revealed the deposition of lignins in lower differentiated xylem cells as compared to the controls. The first labelling of condensed lignin appeared in cell corners and of non-condensed lignin in secondary walls near cell corners during the deposition of S1 polysaccharides. Because of alterations in the formation and differentiation of xylem cells, 35S-*rolC* transgenic aspen may be useful for studies on molecular factors controlling the differentiation continuum.

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Title : TRANSGENIC ASPEN: TRANSGENE INTEGRATION, STABLE EXPRESSION, AND FUNCTIONAL GENOMICS

Abstract : The production of transgenic trees is increasingly becoming an important component of forest biotechnology. For commercial success of tree transgenics, however, the questions need to be addressed which are related to the stable integration of transgenes and the faithful transmission of the introduced traits through successive generations in a predictable manner. The transgene incorporated into the plant genome is integrated randomly and in unpredictable copy numbers, often in the form of repeats abolishing the expression of transgene[1] and sometimes leading to excision of the transgene[2]. The integration site also has a profound effect on expression of the transgene, and insertion of the transgene in or near another gene may cause an undesired phenotype[1].To obtain insight into the mechanism of transgene integration and repeat formation, we screened several transgenic lines of wild (*Populus tremula*) and hybrid (*P. tremula x P. tremuloides*) aspen transformed with six gene constructs through *Agrobacterium*-mediated transformation[3]. The T-DNA repeat analysis was carried out using reverse primer PCR[4]. The genomic regions flanking transgene were determined using Inverse-PCR and genomic target regions were successfully amplified in ten independent transgenic lines containing single copy of the transgene. On the basis of the results obtained a model has been proposed for transgene integration and repeat formation in aspen[5]. We suggested a comprehensive and integrated approach for gene targeting[6], to ensure long-term stable transgene expression in plants, and for knocking out endogenous genes to elucidate their functions. Furthermore, we obtained T-DNA random insertion variants in aspen which are being analyzed for the putative genes. ***References:*** **1.** Kumar S & Fladung M (2001) Planta (in press). **2.** Fladung M (1999) Mol Gen Genet 260, 574-81. **3.** Fladung M, Kumar S, Ahuja MR (1997) Transgenic Res 6, 111-21. **4**. Kumar S, Fladung M. (2000) Biotechniques 28:1128-37. **5.** Kumar S, Fladung M. (2000) Mol Gen Genet 264: 20-28. **6.** Kumar S & Fladung M (2001) Trends Plant Sci 6:155-159.

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Title : CRYOPRESERVATION OF TRANSGENIC SILVER BIRCHES

Abstract : During the last few years, genetic transformation techniques have been developed for several broad-leaved tree species including silver birch (Betula pendula Roth) (Valjakka et al. 2000). Due to the importance of the species for plywood and pulp industry etc. also the possibility for molecular breeding has been looked forward. Cryopreservation i.e. the storage of material at low temperatures, usually in liquid nitrogen, is generally used in broad-leaved tree species for long-term maintenance of specific genotypes or cell lines, and to avoid increased risks of contamination and somaclonal variation due to prolonged tissue culture. These facts are also valid in the case of transgenic trees. In addition, the production of cell lines with desired level of transgene expression or desirable phenotype is laborious and for practical applications the existing lines need to be multiplied. Thus for long-term storage of transgenic lines with high regeneration capacity reliable cryopreservation methods are necessary. The cryopreservation of transgenic silver birch was studied in order to find out the applicability of cryopreservation on the storage of transgenic material with special emphasis on the integrity of the transgenes. The study was performed on two birch clones, R and E5396, with two transgenic lines in both of them. Transgenic lines included neomycin phosphotransferase (npt2) and ribulose-1.5-bisphosphate carboxylase/oxygenase small subunit (RbcS) genes (Valjakka et al. 2000) or npt2 and ß-glucuronidase (uid A) genes (Töpfer R et al. 1988) under 35 S CaMV promoter. For cryopreservation, vegetative branch and stem buds were collected of one-year-old and two-year-old birches grown in greenhouse with natural light conditions and the natural temperature limited to minimum of 5 °C. The buds were cryopreserved (Ryynänen 1996) at least for one week, after which they were cultivated in vitro. The regeneration of buds was estimated visually after two and four weeks of cultivation. After two weeks of in vitro cultivation regeneration of cryopreserved branch buds of both clones was significantly slower than that of the corresponding stem buds. Also regeneration of cryopreserved buds presenting the clone E5396 was retarded when compared to the non-cryopreserved ones. After four-week in vitro cultivation, however, no difference in regeneration was observed. The integrity of introduced genes in transgenic lines before and after cryopreservation has been verified by southern blotting. Northern hybridisation techniques have been used to prove the expression of transferred genes. References Ryynänen L (1996) Survival and regeneration of dormant silver birch buds stored at super-low temperatures. Can. J. For. Res. 26: 617-623. Töpfer R, Schell J and Steinbiss H-H (1988) Versatile cloning vectors for transient gene expression and direct gene transfer in plant cells. Nucleic Acids Res. 16: 8725. Valjakka M, Aronen T, Kangasjärvi J, Vapaavuori E and Häggman H (2000) Genetic transformation of silver birch (Betula pendula) by particle bombardment. Tree Physiol. 20: 607-613.

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Title : TRANSCRIPTIONAL CONTROL OF EUCALYPTUS GENES INVOLVED IN LIGNIN BIOSYNTHESIS

Abstract : One of the major objectives of the paper industry is to optimise wood properties in order to enhance pulp and paper quality, using more environmentally-friendly processes with reduced chemical use and decreased energy consumption. Since wood properties rely mainly on xylem secondary cell wall structure and composition, it is of considerable interest to elucidate the molecular mechanisms underlying secondary cell wall formation and assembly. Toward this end, our lab has focused its efforts on the functional characterisation of the promoters of two specific lignin biosynthetic genes (Cinnamoyl CoA reductase (CCR) and Cinnamyl alcohol dehydrogenase (CAD2) from Eucalyptus. Expression studies during development of tobacco and poplar revealed that the CCR and CAD genes are preferentially expressed in vascular tissues, more particularly in xylem, and that this specific expression is transcriptionally regulated. Futhermore, CAD and CCR promoter regions responsible for this transcriptional control have been identified and shown to be targets for nuclear DNA-binding factors. We are now developing several approaches to characterise transcription factors regulating the vascular expression of these genes. In this respect, progress on the functional characterisation of candidate Myb transcription factors will be presented.

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Title : THE PROMOTER OF A CYTOSOLIC GLUTAMINE SYNTHETASE GENE FROM THE CONIFER *PINUS SYLVESTRIS* IS ACTIVE IN GERMINATING SEEDS AND LIGHT-REGULATED IN TRANSGENIC *ARABIDOPSIS THALIANA*

Abstract : Ammonium is assimilated into amino acids through the sequential action of glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes. We are investigating nitrogen assimilation and metabolism in conifers, using Scots (*Pinus sylvestris*) as experimental model.Here we report the isolation and characterization of a genomic clone encoding Scots pine (*P. sylvestris*) cytosolic glutamine synthetase. The clone contains the 5' end half of the gene including part of the coding region and 980 bp upstream the translation initiation codon. The major transcription start site (+1) was mapped around 180 nucleotides upstream the translation initiation codon. Sequence analysis of the 5'-upstream region of the gene reveals the presence of putative regulatory elements including a poly-CT consensus sequence, a purine-rich tandem repeat and two AT-rich regions. Fusions of the upstream gene region to uidA were shown to be transiently expressed in the cotyledons of germinating pine seeds transformed by microprojectile bombardment. Stable transformation of *Arabidopsis thaliana* revealed the shoot apical meristem as the major region of heterologous permanent expression in *Arabidopsis*, in agreement with the expression of the GS gene in *Pinus*. Moreover, quantitative data derived from fluorometric GUS assays in control and continuous light-grown transgenic *Arabidopsis* plants indicate that the isolated upstream region of the gene contains regulatory sequences involved in the response to light. Funded by PB98-1396, Spanish Ministry of Science and Technology

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Title : GENETIC ENGINEERING OF MARITIME PINE (PINUS PINASTER AIT.)

Abstract : Maritime pine covers more than 4 millions hectares in the Mediterranean region and, with about 1.4 million hectares, it represents the first coniferous species used for reforestation in France. Biotechnology programs implemented by AFOCEL in the past 10 years are expected to significantly speed up the long-term breeding programs initiated in the 1960's. In conjunction with cryopreservation techniques, development and integration of somatic embryogenesis in the improvement process could for instance greatly increase the availability of trees selected according to the requirements of wood chain industries. Moreover, such a promising tissue culture system undeniably provides a good target for genetic engineering and offers new prospects for rapid and efficient introduction of desirable traits, mostly unknown (e.g. herbicide or insect tolerance) or with low heritability in maritime pine (e.g. wood quality and processing, vigor). As a starting point, two different approaches were evaluated to transform embryonal suspensor masses initiated from selected seed families: the microprojectile bombardment technique (biolistic) and the Agrobacterium tumefaciens based method. In both cases, selection of transformed cell lines using the antibiotic hygromycine B was demonstrated to be highly efficient at no more than 10-20 mg/l within 4-17 (biolistic) or 4-19 weeks (Agrobacterium). We used a co-integration procedure of reporter uidA gene without intron (GUS activity) and selective hpt gene (hygromycine resistance) for biolistic experiments, whereas uidA gene with intron (suppressed GUS activity in bacteria) and hpt gene were present within the same T-DNA in Agrobacterium experiments. uidA and hpt were both under control of CaMV35S promoter. Stable integration and expression of reporter and selective genes was observed in 46-67% (biolistic) or 75-89% hygromycine resistant lines (Agrobacterium). In the latter case, Agrobacterium decontamination of most cocultured lines was achieved with 4 weeks maintenance on culture medium supplemented with 300 mg/l Augmentin, without any drastic effect on plant cell growth. Depending on experiment and Pinus pinaster genotype, transformation efficiencies were in the range 1.25-12.25 (biolistic) or 0-153 (Agrobacterium) transformed lines per gram tissue (fresh weight). Mature somatic embryos could be regenerated from several of these lines and transgenic plants expressing the GUS reporter gene in different tissues are currently growing in the greenhouse for further molecular and morphological evaluation. Key words: Pinus transformation, biolistic, Agrobacterium, transgenic tissues and plants

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Title : TRANSFORMATION OF SCOTS PINE AND NORWAY SPRUCE POLLEN RESULTING IN PRODUCTION OF TRANSGENIC PROGENY

Abstract : In many conifers, difficulties in tissue culture are one reason for unsuccessful regeneration of transformed cells or tissues. Pollen, on the other hand, is a natural vector for gene transfer, and regeneration problems could be avoided by using transformed pollen in controlled pollinations. This approach has proved to be successful in tobacco. Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies (L.) Karst) are the most common conifers in the Nordic countries, and the traditional breeding programs and biotechnological studies have been focused on them. For both species a transformation method independent of in vitro regeneration would be favoured. Several reports on the successful genetic transformation of gymnosperm pollen through particle bombardment have been published, and we have reported the first attempts to utilize transformed pollen in crossings. In the present study the effect of different gene constructs on transgene expression in the mature pollen of the most important Nordic conifers - Norway spruce and Scots pine- was studied using particle bombardment. The best gene constructs containing the b-glucuronidase (GUS) as reporter gene were chosen, and bombarded pollen used for controlled pollinations. Originally, a liquid pollination technique was used in both species, owing to necessity to provide target cells for bombardment as suspension. In addition to genetic transformation studies, the compatibility of liquid pollination technique was compared with conventional crossings using dried pollen and with open pollinations in Norway spruce and in Scots pine. In order to assure successful pollinations also after bombardment of pollen, also possibilities to re-dry and store pollen after bombardment for conventional pollinations were studied. The progenies originating in the controlled crossings are grown in greenhouse, and the transfer of GUS gene studied using histochemical assay and specific PCR multiplication. In Norway spruce and Scots pine pollen, high levels of transgene expression (44 and 55 % of the germinated pollen grains, respectively) were achieved using the plasmids pBM113 and pCGUD0 with the wheat em- and sunflower ubiquitin promoters. Liquid pollination technique proved inferior to conventional crossings, and therefore a method was developed for dehydration of bombarded pollen suspensions and their storage -20°C before conventional pollinations. In the histochemical assays performed with leaves or needles some GUS expression has been seen in Norway spruce and Scots pine progenies. In both cases, the blue colour was faint, and the seedlings have further been examined at the DNA level. So far, only part of the progenies has been tested by PCR, and production of one transgenic Scots pine seedling has been confirmed. More progenies will be tested, and the transgene expression followed during subsequent growth seasons.

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Title : THE ROLE OF CYTOKININ PERCEPTION IN THE REGULATION OF CELL PROLIFERATION RELATED TO WOOD DEVELOPMENT

Abstract : Wood and phloem are derived from actively dividing initial cells of the vascular cambium. Thus, understanding the molecular framework related to the activity of the cambial initials has an important contribution to wood sciences. Our group is focusing on the regulation of cambium through a genetic approach in *Arabidopsis thaliana* and a molecular approach in birch (*Betula pendula*). We are focusing on the primary development of the vascular tissue (procambium) in the *Arabidopsis root*. We have shown that the vascular tissue is established through a series of asymmetric cell divisions in the procambium. The pattern of these divisions is analogous to the cambial divisions of wood, suggesting a common mechanistic basis. We have started to search for *Arabidopsis* mutants with an altered cellular organization of procambium. The primary effect of the *wooden leg (wol)* mutation is the lack of the formative cell divisions required for the organization of the vascular tissue (Scheres et al. Development 121: 53). We have determined that the *WOL* gene codes for a putative signal transducer with histidine kinase activity (Mähönen et al. Genes Dev 14: 2938). Recently, Inoue et al. (Nature 409:1060) showed that CRE1/WOL is a true cytokinin receptor. Taken together, our results indicate that cytokinins regulate the procambial cell divisions of the *Arabidopsis* root through a specific signal transduction pathway. The molecular genetic characterization of this pathway is underway. Consequently, we want to investigate whether CRE1/WOL-like receptors likewise regulate cell proliferation in the vascular cambium of a tree. As a support for this hypothesis, we have recently identified three *CRE1/WOL*-like genes expressed in the cambial zone or the root tip of a birch tree. Functional analysis of the genes is underway. We are going to investigate whether we can influence cambial activity of a birch tree by modifying the activity of the CRE1/WOL -like putative cytokinin receptors.

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Title : MOLECULAR GENETICS OF DROUGHT STRESS RESPONSE IN MARITIME PINE

Abstract : We have initiated a study to isolate and characterize the genes/proteins involved in the drought stress response of maritime pine. We are currently employing three different approaches to identify putative drought-responsive genes: 1/ differential screening (cDNA-AFLP) of transcripts from needles (N) and roots (R) of drought-stressed (S) and well-watered (W) hydroponically grown seedlings; 2/ random cDNA sequencing in the four cDNA libraries (NW, NS, RW, RS); 3/ proteome profiling. This study focus on the first approach. To date, we have identified over 80 drought-induced or repressed cDNAs. Within this set are a number of genes commonly observed to be drought-responsive, as well as other genes described for the first time as being involved in the drought-stress response. We will also discuss the use of this information in a candidate gene approach to characterize water use efficiency QTL.

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Title : GENETIC TRANSFORMATION OF *PINUS PATULA* EMBRYOGENIC TISSUE AND SOMATIC EMBRYOS BY MICROPROJECTILE BOMBARDMENT

Abstract : Conifers are renowned for their recalcitrance in tissue culture and to the introduction of foreign DNA. Particle bombardment has provided a successful alternative to indirect gene delivery systems, such as *Agrobacterium*, and has resulted in several transgenic conifers. The production of transgenic *Pinus patula* would have enormous influence on the timber and pulp production in South Africa. Conferred resistance of *Pinus patula* to herbicides would be cost effective to commercial growers, reducing labour intensive silvicultural practices. This project aims to optimize a protocol to introduce herbicide resistance of *Pinus patula* somatic embryos and embryogenic tissue by particle bombardment. Four embryogenic lines are maintained on solid and liquid MSG3 medium and further matured to the embryo stage. Both embryogenic tissue and somatic embryos at stages I, II, III and IV and germinated embryos, are used for the transformation experiments with the pAHC25 plasmid. The pAHC25 construct contains the GUS reporter gene and the herbicide resistance gene *bar*, each under the control of a maize ubiquitin promoter (*Ubi1*). Selection is achieved using solid MSG3 and 240 maturation medium supplemented with BASTA® herbicide. Bombardment experiments were optimized using pre-bombardment medium and higher vacuum and pressure settings. The bombarded cultures were tested for transient expression. Successful transformation indicated the putative stable integration of the introduced genes which can be tested using PCR and Southern Hybridisation

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Title : STABLE PROTEIN 1 (SP-1) FROM POPULUS TREMULA: RELEVANCE TO FOREST TREE DROUGHT AND SALINITY STRESS TOLERANCE AND ITS CHAPERONE-LIKE FUNCTIONS

Abstract : Alleviating the hazards of abiotic stress, especially salinity, drought and extreme temperatures, is one of the major future challenges of forest tree biotechnology. If, concomitantly, a biotechnological product can be obtained from the trees - a clear advantage can be visualized. In the following we describe the isolation and partial characterization of such a protein with dual in vivo and in vitro functions, a boiling-, detergent- and protease-stable protein with chaperone-like activity. Boiling-stable proteins were studies in our laboratory, and a novel 66 kDa boiling-stable protein, BspA, was detected, which accumulated in aspen plant (Populus tremula L.) in response to water stress, cold temperature and abscisic acid application. Using the anti-BspA antibodies, a cDNA was isolated from an expression library, and sequenced. The isolated cDNA encoded a 12.4 kDa novel boiling-stable hydrophilic protein, which is different from the 66kDa protein, and was termed SP-1. Northern blot analysis revealed that sp-1 encodes a small mRNA (about 0.6 kb) which is constitutively expressed in aspen plants, but its accumulation is stimulated considerably by water stress, salt stress, osmotic stress, cold and heat shock. The SP-1 that was detected in plants is stable upon boiling, and was of at least two sizes, as detected on 17% SDS-tricine gel: 12.4 and 116 kDa. Comparative protease digestion patterns, amino acid analysis and N-terminal sequences of the two species of SP-1 revealed that SP-1 is a homo-oligomeric protein. Furthermore, gel filtration chromatography analysis indicated that SP-1 exists in aspen plant as a complex, composed of several, up to fourteen 12.4 kDa subunites. Recombinant SP-1 was expressed in E. coli, and was also found in a complex form. The SP-1 complex is resistant to SDS and protease digestion. The high stability of SP-1 oligomer suggests the existence of unique strong interactions in the oligomer. Studies of the effect of SP-1 on the in vitro protection of citrate synthase and horseradish peroxidase activity from heat-inactivation, demonstrates that SP-1 functions in stabilization of the enzymes. A transgenic aspen clone, transformed with sp-1 cDNA, shows SP-1 over-expression and increased tolerance to high levels of NaCl in pot experiments, as determined by growth parameters. A close protein species was found also in Aleppo pine trees (and in tomato). The expression of this protein (and of other boiling-stable proteins) was used to detect the response of several Aleppo pine provenances to changing climatic conditions (summer drought and winter) under natural forest conditions, as well as seedling subjected to NaCl treatments. To the best of our knowledge, this is the first report describing the isolation, cloning and characterization of boiling stable, stress responsive chaperone-like proteins from Populus, and from plants in general. The isolated SP-1 has a unique homo-oligomeric structure resulting from the assembling of several 12.4 kDa protein units. Funding by the European Union (INCO-IC18-CT97-0200-FORADAPT and QLK5 - 2000 -01377-ESTABLISH), and by Israel-India Biotechnology Research Fund, is gratefully acknowledged

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Title : IDENTIFICATION OF GENES DIFERENTIALLY EXPRESSED IN XYLEM ASSOCIATED WITH DIFFERENT TYPES OF WOOD IN MARITIME PINE.

Abstract : Wood is the end-product of xylogenesis and has a support role for the tree. Six types of wood (early/late, juvenile/mature; reaction/opposite) can be observed within the same tree. Each type of wood possess distinct chemical, anatomical and physical characteristics. We hypothesized that these differences are due to differential gene/protein expression rather than resulting from different developmental programs. The objective of this study was to test this hypothesis in maritime pine, the first confifer species used for reforestation in south-western Europe. We used two mRNA fingerprinting methods (cDNA-AFLP and suppressive soustractive hybridation, SSH) to screen for differentially expressed genes in xylem associated to these different types of wood. A total of 182 fragments were identified, cloned and sequenced. Overall, 38% of the sequences were similar to known function genes (metabolism, cell wall, signal transduction, genes and proteins expression, stress, hormone biosynthesis, cytosqueleton and transport), 24% corresponded to unknown Arabidopsis proteins, and 38% showed no similarity in public databases. The validation of the expression is being performed using cDNA macro-arrays. These genes provide expressional candidate genes that will be localized to the maritime pine genome to investigate whether they coincide or not with wood quality and end-uses properties QTLs. This targeted approach is completed by EST sequencing from a normalized-composite library constructed with xylem associated with these six types of wood.

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Title : IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN MARITIME PINE SEEDLINGS EXPOSED TO WIND TREATMENT

Abstract : The most visible response of trees to mechanical stresses (either static or dynamic stimulations) is the formation of reaction wood, in which ethylene plays an important role. The molecular mechanisms involved in a response to such stress remain largely unidentified. The objectives of this experiment were two fold: (i) to identify the genes which are initially switched on in wind-stressed trees and, (ii) to determine whether ethylene is directly involved in this response or is just a by-product of the mechano-transductive pathway. We used the cDNA-AFLP technique to generate mRNA fingerprints from four samples collected on 1-year old seedlings, obtained by crossing wind treatment (wind vs. no wind) with 1-MCP (1-methylcyclopropene, an inhibitor of the ethylene receptor) treatment. Needles were collected after a few hours of treatment, frozen under liquid nitrogen and stored at -80°C, until mRNA was extracted. A total of 20 fragments were identified as being differentially expressed in at least one of the four conditions. By comparing mRNA profils of wind and no-wind treated seedlings we could identify 12 genes that were involved in the initial thigmomorphogenic response (10 and 2 being up- and down-regulated, respectively in the wind treated plants). By comparing mRNA profiles of seedlings treated or not with 1-MCP, interesting results were found: in the control treatment (no wind) only 3 fragments were regulated, whereas in the wind-treated plants, 16 fragments were differentially expressed between normal and "artificially mutated" plants. This primary result tends to show that ethylene acts as a second messenger in the mechano-transductive pathway

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Title : IDENTIFICATION OF DIFFERENTIALLY ACCUMULATED mA FROM POPLAR DURING TENSION WOOD FORMATION THROUGH AN AFLP-BASED APPROACH

Abstract : With the aim to identify and characterize target genes important for some wood properties we initiated a genome-wide mRNA expression study on poplar wood formation. As a model case, we focused first on the formation of tension wood in response to mechanical stress. Tension wood is formed at the upper face of branches and tilted stems in response to a gravitational stimulus. This tissue is characterized by some distinctive ultractructural, biochemical and mechanical properties, and is known to play an important role in the tree architecture. Using this experimental model we intend to identify genes highly up- or down-regulated at the transcriptional level. For this purpose, AFLP-based transcript profiling was used to compare differentially accumulated mRNA from tension wood to opposite or upright wood . Tension wood formation was obtained on one year old poplar tree grown in the greenhouse and tilted for 2 month. From 96 different couple of enzyme/primer tested we found more than 100 transcript-derived fragments presenting a differential amplification. Approximately 80% of the differentially amplified TDFs were shown to be specifically or mainly amplified from tension wood compared to opposite wood. About 70 of these TDFs have been cloned. For some of them, expression studies by reverse northern and RT-PCR have been carried on. This work is supported by a grant from the " Direction de l’Espace Rural et de la Forêt " (Déterminisme génétique de la qualité du bois chez les arbres forestiers : approche gènes candidats ; convention N° 01.40.40/99). F.L. is indebted to the "Conseil Régional de la Région Centre".

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Title : EPIGENETIC AND PHYSIOLOGIC *Pinus radiata* D.Don. AGEING AND PHASE CHANGE MARKERS

Abstract : DNA methylation and polyamines titter have been analysed during ageing and before and after the radiata pine phase change in order to define possible epigenetic and physiologic markers. Juvenile individuals (without flowering ability) were characterised by a DNA methylation degree of 30-35% and a free polyamines to perchloric acid soluble polyamines conjugates ratio lower than 1, while mature trees (with flowering ability) showed 60% 5-methylcytosine and a free polyamines to perchloric acid soluble polyamines conjugates ratio lower than 1. Results obtained with a clone that started it reproductive development during the research period time confirm that the changes between juvenile and mature states have been demonstrated to occur just after the phase change. We propose that among others, both indicators are related with the lost of morphogenic ability during ageing and specially after phase change through specific interactions that are widely discussed. Financial source: FAIR3-CT96-1445.

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Title : GENES IMPLICATED IN MERISTEM FUNCTION IN WOODY PLANTS

Abstract : The activity of meristematic tissues plays a major role in determining the form and rate of growth of all plants. Great progress has been made in delineating the mechanisms that underlie events related to the establishment, maintenance, and maturation of meristematic tissues in herbaceous annual plants. The analysis of mutants impaired in normal meristem function has uncovered a suite of genes involved in the control of meristem activity in herbaceous annual plants. In contrast, very little is known about the molecular mechanisms that control meristem activity in perennial plants like trees. We have been testing hypotheses aimed at determining if both the function and deployment of genes implicated in the regulation of meristem activity in ephemeral herbaceous annual plants, like arabidopsis, are conserved in long-lived woody perennial plants, like pine and eucalyptus. Consistent with current theories of molecular evolution, analysis of these genes has revealed that, while the function of the gene products is conserved, the regulation of these genes differs between herbaceous and annual species. Beyond this, our studies have suggested strategies whereby these genes might be manipulated to alter tree development for desired end uses.

SESSION 8

Chairs, Beppe Vendramin/ Zeki Kaya

Molecular diversity and application of molecular markers for seed and plant material certification

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Title : ANALYSIS OF CEDRUS GENETIC GEOGRAPHIC DIVERSITY USING AFLPS

Abstract : The Cedrus genus belonging to the Pinaceae family is commonly regarded as comprising four species: C. atlantica, growing in the Atlas and Riff Mountains of North Africa, C. brevifolia, endemic to Cyprus island, C. libani, native to Lebanon, Syria and Turkey and C. deodara, occurring in the Himalayan Mountains. Fifteen cedar populations representative of the different geographical localization of the four species were sampled to assess their genetic diversity using Amplified Fragment Length Polymorphism techniques. Genetic variation was investigated within and among populations of genus Cedrus. A total of 101 polymorphic amplification products were generated using 12 AFLP primer combinations. Nei's genetic similarity estimated within the different populations showed the highest value in the Turkish experimental stand of Elmaly (close to the Antalya region) with 0.29. The lowest intra-population variability was found in the Himalayan population and in a C. atlantica population from Morocco with 0.12 and 0.11 respectively. Levels of differentiation (G_{ST}) were calculated among the different cedar populations tested. The highest G_{ST} values were observed when the Himalayan cedars were compared to the Mediterranean populations. The lowest G_{ST} values were obtained when C. brevifolia was compared to C. libani populations. Nei's genetic distance estimated by UPGMA generated several clusters. At the first level, the Himalayan cedar was clearly separated from Mediterranean cedars. Among the Mediterranean populations, we observed two main clusters, the first one regrouping the 4 C. atlantica populations studied, the second comprising oriental Mediterranean cedars. Within the second cluster, C. libani from Lebanon were separated from Turkey populations. Interestingly the Cyprus cedars were clustered within the Turkish populations. These results support the hypothesis subdividing the Cedrus genus into three species, C. atlantica, C. deodara and C. libani while C. brevifolia is considered as a variety of C. libani.

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Title : A CYCLOPHILIN GENE MARKER AS INDICATOR OF GEOGRAPHIC VARIATION AND GENETIC RESPONSE TO SALT STRESS IN NORWAY SPRUCE

Abstract : A full-length cDNA-clone has been isolated from Norway spruce [Picea abies (L.) Karst.], encoding a 18.266 kDa protein in which 75-77 % of the amino acids positions are conserved relative to the known cyclophilin sequences of higher plants (Zea mays, Lupinus luteus, Catharanthus roseus). Based on this sequence, a PCR primer pair was designed for the amplification of a locus-specific codominant EST marker (PA0005). Following RsaI digestion, the fluorescently-labelled polymorphic product patterns were detected using electrophoresis in high resolution gels. In 13 natural populations, representing a broad European range, a total number of 7 alleles were identified. The predominant frequency of the allele A (504 bp) was confirmed for all populations in contrast to low-frequency alleles C, D, E, F and G which were absent in some populations. The distribution of allele B (526 bp) shows, however, a geographic gradient related to the different postglacial recolonization routes of Norway spruce. Its frequency distribution is range from 8 % to 20 % in the Romanian, Hungarian, Southern Polish, Southern German, Italian and Swiss population samples tested, with highest values found in the Alpine region, while this allele is missing or revealing very low frequencies in the gene pool characterized by test populations from Russia and Norway, respectively. In addition, two closely located Bavarian stands (10 and 30 years old) suffering from sodium chloride-caused soil pollution, were investigated. In these stands, 35 and 36 pairs of trees, respectively, were sampled. Each pair consists of a sensitive tree, showing needle discolorations, and the next uneffected tolerant neighbor. The two-locus genotype AA-BB of the marker combination PA0005 and the recently developed marker PA0066 (targeting to a ribosomal protein gene), reveals frequencies of 28 % and 34 % in the two sensitive subsets in contrast to 58 % and 66 % in the corresponding tolerant subsets. Both homozygous marker genotypes are considered to be indicative of the genetic response to the stress conditions tested. These data demonstrate that the newly developed cyclophiline gene marker offers a tool for the genetic characterization of provenances and furthermore represents a first step towards marker-assisted selection of superior trees with respect to salt stress.

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Title : THE IMPACT OF ANTHROPOGENIC FACTORS ON THE COMPOSITION OF GENETIC VARIATION ON *PINUS BRUTIA* TEN. POPULATIONS DETERMINED BY RAPD MARKERS

Abstract : Randomly Amplified Polymorphic DNA (RAPD) markers were used to study genetic diversity in 6 populations of *P. brutia* from the Mediterranean Region of Turkey to explore the impacts of anthropogenic factors on the magnitude of genetic diversity in the species. Initially, 80 RAPD primers were screened and the best yielding 12 were used to collect RAPD data from three degraded (Alanya-Kargi, Manavgat-Yaylaalan, Antalya-Çalkaya) and three natural (Fethiye-Yapraktepe, Burdur-Gölhisar, and Çameli -Göldag) populations of Pinus brutia. The 12 RAPD markers yielded 231 polymorphic alleles. Four populations studied had 5 unique alleles and 2 of the unique alleles were from a degraded population, Manavgat-Yaylaalan. The mean proportion of polymorphic loci for all populations (0.99 criterion) was 86.29 implying high levels of variability. The estimation of heterozygosity indicated that *P. brutia* exhibits high levels of genetic differentiation ranging from Ho=0.215 in Burdur-Gölhisar to 0.264 in Fethiye-Yapraktepe. Estimated FST value suggests that the highest proportion of genetic diversity was within populations that constitutes 91.45% of the total variation. In degraded populations, there was slightly loss of genetic diversity (5.33%) and F-statistics analysis showed that there is 7.3% higher heterozygosity deficiency in these populations.

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Title : VALIDATION OF CONTROL CROSSES AND DETECTION OF INTERSPECIFIC GENE FLOW AMONG MEDITERRANEAN *CEDRUS* SPECIES USING RAPD, AFLP AND CPSSRS

Abstract : Interspecific controlled crosses were performed on *Cedrus atlantica* seed parents using pollen from the 3 Mediterranean *Cedrus* species: *C. atlantica* from North Africa, *C. libani* from the Middle East and *C. brevifolia* from Cyprus. The experiments were conducted within two different populations of *C. atlantica* in southern France where this naturalized forest species is commonly planted because of its adaptation to mid-elevation Mediterranean conditions and high quality timber. Out of the 64 pollinations performed, only 3 yielded viable seeds. These controlled progenies and the corresponding open pollinated progenies from the same female parents were analyzed using 3 classes of molecular markers: RAPD, AFLP and chloroplast microsatellites (cpSSRs). The main results of the study were as follows: - Hybridization among the 3 species is possible. - RAPD and AFLP analyses jointly showed that two of the progenies resulted from interspecific crosses while the 3rd contained only maternal type seeds, indicating the absence of interspecific hybridization. When used separately, RAPD and AFLP analyses could not unambiguously identify true controlled progenies. - Chloroplast DNA is paternally inherited in the genus *Cedrus*. - cpSSRs unambiguously identified hybrid seedlings in the controlled pollinated progenies. - *C. libani* cpSSR variants (interspecific hybrids) were detected in the open pollinated seed crop of the *C. atlantica* seed trees in both populations. Interspecific geneflow accounted for approx. 3% of viable seedlings obtained in one of the *C. atlantica* populations. No *C. libani* pollen donors could be located in the vicinity of the two female trees tested. In the other population, *C. libani* pollen donors were within a few meters of the only female tree tested which yielded more than 80% hybrid seedlings. Interspecific geneflow can be of variable magnitude in southern France *C. atlantica* populations. When these have been designated as reference stands for seed collection, the use of cpSSRs should be recommended to monitor potential genetic pollution within annual seed crops. These markers could also be used to select candidate populations for genetic conservation purposes. Finally, cpSSRs can be successfully used for species identification and seed "purity" certification in the genus *Cedrus*.

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Title : USE OF CHLOROPLAST MICROSATELLITE MARKERS FOR THE ANALYSIS OF GEOGRAPHIC DISTRIBUTION OF DIVERSITY IN *PINUS PINASTER* AIT.

Abstract : Haplotypic variation within and among populations of Maritime pine (Pinus pinaster Ait.) was evaluated using six polymorphic chloroplast microsatellite (cpSSRs) markers. About 50 populations, each represented by at least 20 individuals and sampled in the different part of the natural range, were analysed with the main objectives i) to obtain information about the history of this species during the last glaciation and in the post-glacial period; ii) to use the information about the geographic distribution of cpSSRs diversity for the development of efficient tests of seed and provenance certification. The genetic parameters estimated from cpSSRs data revealed that the level of genetic diversity in Pinus pinaster is very high. The estimation of genetic differentiation among populations showed that the majority of the diversity is distributed within populations. A clear and significant differentiation among groups of populations of different geographic origin (Portugal, France, Italy, Spain and Morocco) was observed, while, on the contrary, the degree of population divergence within country is generally very low and close to zero thus indicating a homogeneous distribution of the variation within groups. The presence of significant differentiation among groups of populations of different geographic areas seems to reflect the recolonisation processes in the post-glacial period starting from the different refugia. On the other hand, the absence of a clear and discernible geographic genetic pattern for the populations within some countries (e.g. Portugal and France) may be the result of the intense human activity through large reforestation mainly performed using genetic material of unknown origin. The test for population differentiation showed a clear difference between the French and Portuguese populations (with no differentiation among populations within each country) thus allowing the two provenances to be clearly identified. On the basis of this observation, a new and highly efficient test employing cpSSRs for the identification of stand origin was developed. The test based on cpSSR data was more accurate and more efficient, in term of costs and speed, than other biochemical (e.g. terpene) tests. In conclusion, the cpSSR study performed on Pinus pinaster allowed to describe in details the distribution of diversity in this species and also to gain knowledge about the history of this species. This information can be used for the development of efficient programme of conservation of the genetic resources of this species. Moreover, knowledge about the geographic distribution of diversity was essential for the development of a test for the identification of the origin of the stands. Considering that cpSSR primers cross-amplify sequences of several conifer species, generally producing highly polymorphic fragments, the test can be easily transferred and applied to other commercially important species.

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Title : WHAT CAN NUCLEAR MICROSATELLITES TELL US ABOUT MARITIME PINE GENETIC RESOURCES CONSERVATION AND PROVENANCES CERTIFICATION STRATEGIES?

Abstract : Maritime pine (Pinus pinaster Ait.) is the first conifer species used for reforestation in France, where it covers 1.3 Million ha. In the Iberian Peninsula (Spain and Portugal), the species covers 2.4 Million ha, being 600.000 of them of natural origin. Natural genetic resources of this economically and ecologically important species are now threatened in France : in the long term in the south west (Aquitaine region) by the introduction of seedlings from improved seeds (genetic pollution), and in the short term in the south east of France and in Corsica by the decay due to the bast scale Matsucoccus feytaudi Duc. In addition, mediterranean forests of maritime pine, that typically have low effective population sizes, are under a strong human impact due to forest fires and reforestation with plants of unknown origin. In order to preserve the genetic resources of this species prior knowledge of its diversity level is needed. A genetic diversity study was performed using nuclear Simple Sequence Repeats (SSR). Classical parameters of diversity (allelic richness, heterozygosity) and differentiation were estimated on 46 natural populations. These were collected in the whole range of distribution of Maritime pine (39 from France, 4 from Spain, 2 from Portugal and 1 from Morocco). Population genetic statistics showed that some populations could be interested for conservation purposes (higher level of diversity, higher allelic richness, presence of rare alleles). The result of this analysis also provided a diagnostic test for seed origin certification. We will examplify this possibility in the particular case of Corsican x Aquitaine hybrid certification.

SESSION 9

Chairs, Christine Cahalan / Wout Boerjan

Genome analysis, Conclusions and Perspectives

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Title : ISOLATION OF DNA FROM DRY WOOD: APPLICATON FOR TRACING THE GEOGRAPHIC ORIGIN OF OAK WOOD USED BY THE FRENCH BARREL INDUSTRY

Abstract : Until recently, dry wood, which represents a huge biomass on earth, was not considered as a potential source of DNA. The recent demonstration in our laboratory that chloroplast DNA could be successfully recovered from dry wood using the Polymerase Chain Reaction prompted us to test more systematically the potential for DNA analyses and characterisation on this material. DNA isolation and amplification was carried out on a set of eight different oak logs. The conservation of DNA in different parts of the log, from the bark to the heartwood, was tested by amplifying DNA fragments of increasing size. The usefulness of the three plant genomes as a source of DNA in dry wood was then tested by using primer pairs specific of the plastid, mitochondrial and nuclear genome. The eight logs were of different ages and had been conserved differently, allowing us to monitor DNA conservation through time and to compare different environmental effects on the preservation of DNA molecules. Stringent procedures to avoid and monitor contamination with external DNA were used in order to demonstrate the authenticity of the results obtained. The main goal of this work was to identify molecular markers suitable for genetic analysis on oak wood material, and to understand their limitations. In European white oaks, a detailed geographic map of chloroplast DNA variants has been established during a previous european project involving 12 european laboratories. Trees from more than 2,600 oak forests have been typed during this project, all over the european continent. The strong cpDNA geographic structure that was detected allows to discriminate several regions or countries from each other with these markers. We adapted the cpDNA typing method used with DNA isolated from fresh leaves to the much more degraded DNA remaining in dry wood, by designing primers amplifying shorter but informative cpDNA regions. This procedure allows us to confirm (or to reject) the presumptive geographic origin of a wood lot used by the barrel industry, leading to a better control of the trade, through a procedure of certification.

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Title : MOLECULAR MARKERS AND THE STRUCTURE OF THE GENOME OF NORWAY SPRUCE

Abstract : Different strategies have been used for the development of molecular markers in Norway spruce (*Picea abies* Karst.) in order to cover all portion of the genome, from highly repetitive to single-locus, and/or expressed, regions. In particular, S-SAPs were designed to target retrotransposons, which constitute an abundant class of interspersed repeated sequences; SSRs were designed from randomly selected clones and from clones belonging to the low-copy fraction of the genome; *Eco*RI-*Mse*I AFLPs were used as a random sample of the genome; *Pst*I-*Mse*I AFLPs were used for their preference for undermethylated, and therefore potentially expressed, sequences; EST-based SNPs and other EST-based markers were developed to account for variation within expressed regions. Sets of markers from all classes were mapped in a genetic linkage map, and we will discuss the marker classes distribution in the genome. Microsatellite sequences were found to be sometimes associated with families of repetitive elements, mostly retrotransposons, despite the selection for low-copy sequences. Levels of variability differed across marker classes. Retrotransposon-based S-SAP and AFLPs displayed similar levels of polymorphism. Microsatellite diversity was found to be inversely related to the length of the repeat unit, with considerable variability across loci. Relative estimates of mutation rates of di- and tri-nucelotide SSRs, are obtained from a screening of microsatellites on a sample of approximately 2000 individuals. EST-based markers turned out to be the least variable group so far, with a lower frequency of point mutations than in other plant species, which is somehow in contrast with the results obtained screening spruce populations with isozymes. Sequence polymorphism was observed in all classes of DNA markers. DNA fragments corresponding to S-SAP markers were cloned and sequenced, to verify what fraction of polymorphisms may be due to retrotransposon mobility. The results show that polymorphism in these markers is mostly caused by mutations in the regions flanking the retroelements, and therefore the insertion events presumably predate the sequence polymorphism. This is in accordance with the observation that, in other species, high copy number retroelements are seldom actively replicating. Microsatellite alleles were also sequenced, and high levels of sequence polymorphism were observed, both within and around the repeats. An estimate of the relative age of point mutations and insertions/deletions, on one hand, and of microsatellite expansion, on the other hand, will be given for some loci. The structure of trinucleotide microsatellites was scrutinised in details, showing that some of the sequences might belong to genes. Low levels of polymorphism (expressed as number of point mutations per nucleotide) were observed in ESTs, although in presence of a level of haplotypic diversity comparable to what is found in other species and in other markers. The data presented here are used to draw inferences on the history of conifer genomes and populations.

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Title : IMPLEMENTATION OF SOMATIC EMBRYOGENESIS IN CLONAL FORESTRY: TECHNICAL REQUIREMENTS AND DEPLOYMENT STRATEGIES

Abstract : Cloning of trees using somatic embryogenesis (SE) could have a major impact on tree breeding and commercial plantation forestry. The most important advantage of cloning trees by SE is that embryogenic tissue (ET) can be cryopreserved indefinately in liquid nitrogen. Consequently, this offers an opportunity to develop highly valuable clonal varieties by thawing and re-propagating cryopreserved clones after genetic testing has shown which are the best performers. Commercial deployment of such genetically tested, highly productive genotypes in clonal forestry will drastically increase forest production over any available conventional tree improvement techniques. The successful implementation of SE in clonal forestry requires sufficient technical advances. Obviously, it requires a high SE induction and subsequent plant conversion rates. It is also required that the SE-derived trees are genetically stable, particularly during the cryogenic storage. The development of automated somatic embryo handling system, such as artificial and encapsulated seeds, is desirable although alternative methods can be used. For several conifer species, sufficient advances were made to implement SE in tree improvement programs. The progress in SE process and genetic stability of clones, using white spruce (Picea glauca) and eastern white pine (Pinus strobus) as model species, is presented. There are several issues in deploying clones in plantation forestry. The main concern is bout the management of clonal plantation for optimal genetic gain and diversity. There is a general consensus that the deployment of 15-30 clones in clonal mixtures or monoclonal mosaics would provide protection against catastrophic plantation failure. The issues and considerations for selecting appropriate numbers of clones and deployment strategies are discussed. A clonal deployment strategy, namely ‘wide-mix,’ is proposed for eastern Canada.

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Title : GENE DISCOVERY AND EXPRESSION ANALYSIS FOR LOBLOLLY PINE EMBRYO DEVELOPMENT

Abstract : Embryogenesis proceeds as the result of a controlled program of gene expression. Understanding of embryogenesis is thus assisted by the identification of genes expressed during embryo development, and determination of the function of their encoded proteins and the timing and location of gene expression. Global gene expression assays such as differential display and DNA arrays, in both zygotic and somatic embryos revealed that roughly 5% of genes are actively regulated during embryogenesis. Of the actively regulated genes, approximately one quarter are regulated similarly in somatic and zygotic embryos. The study of genes differentially expressed early in development or of conifer homologs of key genes in angiosperm embryogenesis may shed light on the obscure event of early pine embryo development. One such gene is a novel cDNA (LPnlm1) similar to Nodulin-like membrane intrinsic proteins, a class of the water-transporting Aquaporins. LPnlm1 transcripts are present at the earliest stages of embryogenesis and not detected during embryo desiccation or in seedlings. Uptake assays with transgenic yeast expressing the pine LPnlm1 cDNA confirm that it is a member of the glycerol-aquaporin family of MIPs. In separate studies, differential expression of several storage protein encoding genes in the suspensor tissue of zygotic embryos provide insights into the physiological role and developmental pathway characterizing the suspensor of pine embryos. Finally, in a study focused on pine homologs of key embryogenesis control genes in Arabidopsis, full-length cDNAs for a LEC-like, PKL-like and FIE-like genes have been isolated and expression at different stages of pine embryogenesis has been determined.

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Title : TOWARDS ASSOCIATION STUDIES IN FOREST TREES: WOOD PROPERTY QTL VERIFICATION, CANDIDATE GENES, AND SNPs IN LOBLOLLY PINE (*PINUS TAEDA* L.).

Abstract : By examining the joint distribution of candidate gene genotypes and wood property phenotypes in natural populations, genes controlling wood quality, and the effects of their alleles, can be identified. Our laboratory has initiated such an association study within a sampling of approximately 450 unrelated loblolly pine trees. Which candidate genes to focus on is determined by three criteria; 1) known function, such as in wood and cell wall synthesis, 2) co-localization on genetic maps with quantitative trait loci (QTLs) effecting early- and latewood specific gravity, percentage volume of latewood, microfibril angle, and a host of chemical wood properties (these QTLs were identified in previous experiments and their existence subsequently verified in two larger mapping populations), and 3) gene expression profiles from differentiating xylem of normal versus compression wood. Haplotype classes, defined by a series of single nucleotide polymorphisms (SNPs), are revealed by DNA sequencing of fragments amplified from the 5' and 3' ends of candidate genes using 24 unrelated megagametophytes. Genotyping individuals of the association population at these SNPs is performed by pyrosequencing. Phenotypic data similar to that used in wood property QTL analyses has been collected from the association population. Significant differences in phenotypic means among candidate gene haplotype classes should identify candidate gene alleles with the greatest effect on wood property traits, and will be of great interest to tree breeders. Progress to date will be presented.

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Title : CAD, A CANDIDATE GENE FOR IMPROVING GROWTH AND REDUCING PULP MANUFACTURE COST IN LOBLOLLY PINE

Abstract : The identity of genes for quantitative phenotypic effects (i.e., QTLs) are difficult to determine because QTLs localize to chromosomal regions of 20 to 40 cM, about ~ 1 to 2% of the genomic map length. Such regions could contain 300 to 500 genes. However, some genes are good candidates based on knowledge of their roles in biochemical or regulatory pathways. The best example of a gene that has effects on wood properties is cad, which encodes the enzyme cinnamyl alcohol dehydrogenase. Here, we discuss cad as a candidate gene and describe its phenotypic effects and genetic variation. In the Atlantic Coastal Plain Provenance, the nucleotide diversity of cad was 0.069 in a sample of 23 haploid sequences. The several haplotypes that were identified fall into 2 major clades. Neutral evolution was rejected as an explanation for the deeply divided gene tree. The ratio of nonsynonymous to synonymous substitutions was 2, suggesting balancing selection. The cad n1 allele has almost no gene expression. The phenotypic effect of cad n1 in homozygotes was a dramatic change in lignin composition. While the homozygotes have poor viability, the cad n1 heterozygotes show superior growth. Most loss of function mutations are completely recessive, but the cad n1 heterozygotes had different lignin bonding patterns from normal trees. The wood from the heterozygotes also delignifies more rapidly to a lower kappa and therefore required less energy input for pulp manufacture. These findings demonstrate the potential value of a candidate gene approach for improvement of commercially valuable wood properties and provide a compelling case for a systematic search for other genes that affect wood properties and growth.

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Title : GENOMICS OF EUCALYPT WOOD

Abstract : Eucalypt plantations throughout the world have been primarily harvested for pulp but increasingly the aim is to produce higher value products such as solid wood products. A major focus of our research has been on using molecular technologies to breed for high value wood and fibre traits. Where possible the aim is to integrate these approaches into a breeding program so that gains can be maximised by deployment through clonal forestry. One approach has been to use genomic maps to locate and characterise QTL that control wood and fibre traits. A comprehensive generic map for eucalypts has been developed that consists of codominant markers that can be assayed across the major commercial species. Also on the map are candidate genes for traits such as flowering and wood and fibre traits. QTL have been characterised for wood density, fibre length, pulp yield and microfibril angle. A number of these have been validated in related pedigrees. Research is now concentrated on incorporation of this information into selection strategies. A limited number of QTL collocate with potential candidate genes. Research is now focussed on sequence variation in different parts of these genes and examining if this variation is in any way related to variation in wood properties within the main commercial species. Of particular interest are genes involved in fibre development and cell wall formation. A third approach is to profile the expression of genes in the tissue of interest and determine if this relates to trait variation. Microarrays are being used to relate expression of several thousand genes in xylem tissue to variation in traits such as microfibril angle and density. This will enable detection of not only known genes involved in the control of these traits but also novel genes.

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Title : A CANDIDATE GENES APPROACH IDENTIFIES CCR, PAL AND C4H AS LOCI FOR SYRINGYL / GUAIACYL RATIO IN A INTERSPECIFIC HYBRID BETWEEN *E. UROPHYLLA* AND *E. GRANDIS*.

Abstract : The Eucalyptus breeding programme managed by CIRAD in the Congo has and is still based on improvement of trees growth, form and wood quality. The advanced made in molecular biology and analytical chemistry have made it possible to introduce new selection criteria such as lignin content and quality. For paper-maker industry the elimination of lignins is a very expensive and polluting process. In Eucalyptus species, the lignins average content vary between 20 and 25 %. Also lignins with high proportions of syringyl units (S) are removed more easily during the kraft pulping process. In this study, we report on the identification of genomic regions (QTL) controlling S/G (syringyl/guaiacyl) ratio and on the co-location between these QTL (quantitative trait loci) and genes of the lignification pathway. A full sib family of 201 interspecific hybrids between E. urophylla and E. grandis was used. Eight genes of the lignins biosynthesis pathway [phenylalanine ammonia-lyase (PAL), caffeic acid 3-O-methyltransferase (COMT1 and COMT2), 4-coumarate CoA ligase (4CL), cinnamate 4-hydroxylase (C4H), caffeic CoA 3-O-methyltransferase (CCoAOMT), cinnamyl alcohol deshydrogenase (CAD2), cinnamoyl CoA reductase (CCR)] and three Myb transcription factors (Myb1 Myb 2 and Myb 4) were mapped using the single strand conformation polymorphism (SSCP) technique. These genes were located on the two parental genetics maps constructed with PCR-based markers. The lignins monomeric composition (S/G ratio) were obtained using the thioacidolysis method. The QTL analysis for S/G ratio was performed using the interval mapping procedure. Several regions controlling part of the variation for the S/G ratio were identified. Multipoint estimates of the total variation explained by the QTLs were 38 % and 18,5 % for E. urophylla and E. grandis, respectively. Three co-location between the QTL and the genes were identified for CCR, PAL and C4H. The studied population presented an average S/G ratio of 3.96. A combination of 2 favourable alleles of CCR and PAL or CCR and C4H increases the ratio to 4.35 and 4.36, respectively. This result show that it is possible to manipulate lignins quality by using molecular markers (genes of the lignification pathway),in order to tailor lignins more adapted to specific purposes. Such a markers assisted breeding should provide important economical benefits for utilization of wood in the pulp industry.

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Title : XYLEM GENE EXPRESION, SSR AND EST MAPPING IN *PINUS RADIATA*

Abstract : We are studying gene expression in developing wood tissues of *Pinus radiata* using a modified differential display technique developed at Forest Research (CAGE, Complete Analysis of Gene Expression). DNA sequencing, allele-specific primers, and genetic analysis of CAGE fragments were used to identify differentially expressed alleles. In one example, an allele was expressed more strongly in the cambial/develolping xylem (cdx) tissue from juvenile wood of a heterozygote, while the other allele was expressed more strongly in cdx tissue from mature wood of the same tree. These and prior results also indicate that CAGE methodology can be generally used to detect not only genic but also allelic differences in gene expression. Much is known about differential expression patterns among genes during development or between tissues, but differential patterns of expression between alleles of a gene is not widely documented. Allele-specific expression has important implications for functional genomics, gene engineering, and perhaps even tree breeding applications. The results open the possibility that the 'selective advantage' provided by some alleles in certain environments may be due not just to sequence differences within the gene itself, but also to differences in the level and timing of expression of particular alleles. As part of our QTL detection and comparative genome mapping research we are developing markers and mapping microsatellite (SSR) and EST loci in pines. To efficiently map EST loci we have developed protocols for automated EST allele detection on the ABI 3100 capillary electrophoresis platform. We have mapped about 270 SSR and EST loci in *Pinus radiata*, and a subset of these loci are being mapped in a public loblolly pine pedigree. The current radiata pine codominant marker genome map will be presented.

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Title : COMPARATIVE QTL MAPPING IN CONIFERS

Abstract : Genetic maps have been constructed for a number of conifer species for the purposes of mapping quantitative trait loci (QTL). With just a few exceptions, such QTL mapping experiments lack verification, making it difficult to assess reliability of results. One approach to verify QTLs is through comparative mapping by identifying QTLs mapping to similar locations across pedigrees and species. We have established an international collaboration to construct comparative maps in conifers called the Conifer Comparative Genomics Project (CCGP) (http://dendrome.ucdavis.edu/Synteny). The collaboration is mapping orthologous expressed sequence tagged polymorphism (ESTP) markers derived from loblolly pine cDNAs to a number of pine and other conifer genetic maps. The constituent maps are often populated with QTLs for a number of traits important in forestry; e.g. wood property, growth and adaptive traits. It is now possible to conduct comparative QTL mapping analyses through database queries. Such analyses begin to reveal orthologous QTLs and become logical targets for candidate gene mapping and ultimately cloning.

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Title : GENETIC ANALYSIS OF DISEASE RESISTANCE IN *POPULUS*

Abstract : Poplar is one of the most planted tree species in Europe. Due to the long generation times of trees, the genetic improvement of trees by conventional breeding lags far behind that of annual plants. The possibility to generate genetic maps allows to unravel the genetics of particular traits and to identify the genes behind these traits. This new information can be of great value to improve classical breeding programs and opens possibilities to further improve elite genotypes by genetic engineering. Three genetic maps of poplar (P. deltoides, P. trichocarpa and P. nigra) have been constructed using the two-way pseudo-testcross strategy in combination with AFLP. The maps were generated from 2 controlled crosses sharing the same female parent (P. deltoides cv. 'S9-2' x P. nigra cv. 'Ghoy' and P. deltoides cv. 'S9-2' x P. trichocarpa cv. 'V24'). Microsatellite markers (SSR), made available through the Poplar Molecular Genetics Co-operative (PMGC), were used to align the three maps. These maps are now used to study the genetics of disease resistance, the major selection criterion for poplar breeding. Melampsora larici-populina is one of the most damaging fungal pathogens for poplar in Europe, and both quantitative and qualitative resistance have been recognised. Molecular markers, associated with qualitative resistance to M. larici-populina have been identified and a fine-map has been constructed around the resistance gene as a start point for positional cloning. For this, a binary BAC library has been constructed from a hybrid containing the resistance gene. To reveal loci possibly involved in polygenic resistance, BACs containing disease resistance (NBS-LRR-class) gene-like sequences were identified by BAC library hybridisation, and are currently being mapped. In parallel, QTL analyses for polygenic resistance are carried out. The sequence of one BAC, flanking the M. larici-populina resistance gene, has been determined and annotated, and reveals that the MER genes are present in a large cluster of NBS-LRR type disease resistance genes.

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Title : INCORPORATING WOOD QUALITY TRAITS INTO TREE BREEDING PROGRAMS: CHALLENGES AND OPPORTUNITIES

Abstract : During the last a few decades, selection for tree growth has been the focus of most tree breeding programs. With the worldwide move in forest management to short rotations and the increasing proportion of the wood supply coming from improved and intensively managed plantations, wood quality has become a major concern to the forest products industry. Faced with this concern, many believe that wood quality should form an integral part of tree breeding programs where wood is to be the end product. To this end, numerous scientific research has been done on the genetics of wood quality in many commercial timber species around the world. To incorporate wood quality traits into tree breeding programs, however, there are still some challenges to address. In this paper, the major challenges are reviewed, and potential solutions and need for future research are discussed.

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Title: COMMERCIAL DELIVERY OF GENETIC IMPROVEMENT TO CONIFER PLANTATIONS USING SOMATIC EMBRYOGENESIS

Abstract: Genetic improvement of coniferous trees involves the identification of superior individuals from wild populations (so called plus tree selection) followed by successive generations of breeding and selection of progeny generations. For the major commercial species, including loblolly, radiata and other pines, Douglas fir and several spruce species, breeding has advanced to the point where second generation selections are used for seed production. Elite parental populations exhibiting volume gains in excess of 25% over the base population are often available and improvements in form wood quality and pest resistance have also been made. Historically seed orchards have been used as the delivery system for such genetic improvement. This system is limited by the time required to bring new genotypes into production (up to 12 years). Also, the genetic value of the seed produced is typical less than expected due to uneven gametic contributions and pollen contamination. To mitigate these losses cuttings propagation has been used to multiply control pollinated seed produced in specialized orchards, particularly in radiata pine. In future considerably greater improvement would be possible if true clonal forestry were available in coniferous trees. Conventional cuttings propagation is not suited to the sustained clonal propagation of individuals (clones) in most coniferous species because of physiological aging, which leads to reduced growth rates, aberrant form and poor rooting. The selection and mass production of elite clones offers the opportunity to achieve much greater gains in growth (in the range of 40-60% volume improvement over unimproved) as well as the benefits of uniform wood quality and management and processing efficiencies.

Somatic embryogenesis of conifers has been the subject of intensive research by a number of organizations over the last 15 years. It allows the maintenance and storage of individual trees in a juvenile form for prolonged periods as embryogenic (embryo forming) cultures. In addition, the production of embryos, as opposed to shoots or cuttings, is a prerequisite for bulk production with minimal handling costs (artificial seed). CellFor Inc. is an example of company formed to commercialize this technology with the aim of providing mass production of elite clonal material. To do this approximately 5,000 embryogenic clones of various species have been established for field testing in many locations to select elite individuals of the correct pedigrees for production purposes. In parallel a production system has been developed to enable delivery of the selected clones to operational field planting at an acceptable cost.

CellFor's production system includes large-scale embryo maturation in bioreactors, bulk harvesting, purification and drying of somatic embryos and direct seeding to production nursery environments. These steps and the biological variables to produce embryos of suitable quality for drying and vigorous germination will be discussed.

Currently CellFor is engaged initial commercial production of up to 2 million seedlings per annum and initial field data on trials in several countries is being collected. In future the company expects to introduce several transgenic traits which can be delivered to the market using the somatic embryogenesis production process.

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Title : A GENOMIC APPROACH TO WOOD FORMATION IN LOBLOLLY PINE.

Abstract: We have begun to identity important genes that affect the properties of wood using a genomic approach. In addition to leading to a greater understanding of the molecular basis of wood formation, these studies may establish the basis for use of specific candidate genes to accelerate breeding for specific wood properties. We expect to identify many genes that may be used directly for genetic engineering. We have identified by sequencing partial cDNAs (ESTs), large numbers of expressed genes from wood forming tissues of loblolly pine, and have begun to learn about their functions. We have sequenced 44,000 ESTs from a set of libraries obtained at different stages of normal growth, under mechanical stress, or during a specific season of the year. We have established a unigene set of about 15,000 genes. This set represents about 9,000 singletons and about 6000 that have been identified many times. The expressed genes identified many times provide overlapping sequence (contigs) that often represent near full-length sequence of the mRNA. From these cDNA contigs, we find 9% of the sequences that are potentially unique to pine and its relatives. The contigs also provide information on nucleotide sequence variation, allowing identification of single nucleotide polymorphisms (SNPs) and broad estimates of nucleotide diversity.
Differentiating xylem is a tissue of high complexity with many genes showing differential expression, particularly genes associated with cell wall biosynthesis, such as genes involved in lignin biosynthesis, carbohydrate metabolism, and one carbon metabolism. Several arabinogalactan proteins are abundant and differentially expressed. We have used microarray analysis to compare tissue specific expression and the response to several types of stress, particularly, heat stress and water stress. A group of heat shock response genes were highly expressed in juvenile wood tissue. These genes are not expressed in juvenile wood as part of a generalized stress response, but must have a specific but yet unknown role in this tissue. Using appropriate experimental design and statistical analysis, resolution of differential expression can be far more precise than that of the two fold variation typically estimated in microarray experiments.

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Title : GENETIC AND MOLECULAR DETERMINISM OF WOOD QUALITY IN MARITIME PINE

Abstract : Wood properties are known to vary between species and among genotypes within species. This variability is heritable and thus presents an opportunity to select for improved wood properties i.e. superior product quality. Such selection is currently hampered by costly traditional chemical and technological assays and the necessity to wait until the trees are nearly mature to evaluate wood properties. Application of modern genomic sciences to identify the genes controlling these properties, should increase selection efficiency and/or reduce the time and costs associated with measuring such properties, by providing early selection criteria. In order to provide the maritime pine (Pinus pinaster Ait.) breeding programme with this new strategic option we localised QTLs involved in wood quality traits (lignin quantity and quality, cellulose and hemicellulose content, fibre morphology and pulping characteristics, microdensitometry). Candidate genes/proteins involved in the biosynthesis and assembly of primary and secondary wall components are being identified using the new techniques of transcriptomics and proteomics. A linkage map was constructed to localize these candidates and to investigate if expressional candidate genes also correspond to positional candidate genes, i.e. if they colocalise with the detected QTLs. Reverse genetics in maritime pine and Arabidopsis and linkage disequilibrium mapping are also used to validate putative candidate genes.

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Title : WOOD QUALITY IN BIRCH: CHARACTERISATION AND VALIDATION OF WOOD PROPERTIES FOR INDUSTRIAL USE AND FUTURE BREEDING

Abstract : The main target of the project is to improve the quality of birch wood available to the pulp, paper and board industries through an understanding of wood properties and development of molecular markers associated with specific wood quality traits. This will lead to more efficient breeding methods for wood characters; estimates of genetic correlations involving wood characters; trials and archives of trees with different wood characters, and perhaps also an improvement in the quality of wood available to meet future demand. The tested selection procedures comprise micropropagation, molecular markers, anatomical analysis of the wood, and properties for the pulp and board industries. Trees growing in the widely different conditions prevailing in Sweden, Finland, Germany and the United Kingdom are being examined by the seven partners from five different member states.

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Title : NON-DESTRUCTIVE MEASUREMENTS OF FIBRE LENGTH IN SCOTS PINE

Abstract : Fibre length in Scots pine *Pinus sylvestris* L. can be measured using 5 mm increment cores. This non-destructive method appears to be well suited for analysis of genetic tests. Since many fibres will be damaged in 5 mm increment cores, one may suggest that it is less efficient to use such samples when measuring fibre length compared to using samples from discs or much thicker increment cores. However, disc sampling is destructive, and increment core size must be adapted to the size of the tree in order to be non-destructive. Sampling 5 mm cores is a rapid method, which will allow for large-scale and non-destructive sampling from young trees. In our study, we have compared fibre lengths in annual rings obtained from 5 mm cores with fibre lengths in corresponding large pieces of wood from stem discs. By automatic analyses with Kajaani FiberLab 3.0 equipment, a high number of fibres could be measured. Average fibre lengths were then calculated from the resulting length frequency distributions, and the agreement between average lengths in increment cores and averages obtained from larger wood pieces was high (r=0.87). We thus conclude that 5 mm increment cores are sufficient for ranking and genetic analysis. The method is now used to determine genetic parameters in a Scots pine full-sib progeny test. It is a part of a research program concerning wood properties at the Swedish University of Agricultural Science in Umeå, in collaboration with the Forestry Research Institute of Sweden (SkogForsk), Sävar/Umeå. The poster will show: *i*) histograms of fibre lengths with discussion of data treatment considering the fragmentation due to small increment core radius, *ii*) the connection between data from 5 mm cores and data from adjacent larger wood pieces, *iii*) differences between annual rings, and *iv*) the reliability of repeated FiberLab 3.0 analyses of the same fibre suspension.

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Title : TAKING INTO ACCOUNT THE INTRA-TREE VARIABILITY IN BREEDING PROGRAMS

Abstract : The development of breeding program for wood production as an industrial material and sampling strategies that is linked involve the understanding of the patterns of variation of the wood properties of interest. Information on the variation of important properties across sites, and between and within trees, is essential to obtain the best commercial advantage from the plantation resource. For a lot of species with initial fast growing characteristics, the variability of wood quality traits is significantly greater within each tree than between trees. For technical and commercial reasons, as much as the intrinsic value of a given wood characteristic, homogeneity factors for properties of interest should be introduced in breeding program. This established fact leads to find suitable answers to the questions : how is it possible to obtain an accurate estimation of genetic parameters for traits of interest presenting individual strong variability within tree for an effective selection ? In order to illustrate some possibilities allowing the integration of homogeneity factors into breeding program, we present some results on the variations of some wood properties on eucalyptus clones and within a full-sib family of a hybrid Eucalyptus (E. grandis x E. urophylla). This family comes from the second generation of the plant breeding programme leaded by CIRAD/UR2PI in Congo Brazaville. 200 trees are assessed for several major properties for practical use among which modulus of elasticity, shrinkages and density. All the measurements are performed at the same height (2m), they are sampled along a diameter of the stem. This procedure allows to compare the pith-to-bark variation of the properties between the trees, mainly due to the juvenile phase. The study of the variation in properties across a diameter mean that the traits of juvenile wood can be determined by adjusting parametric curves and by analysing the parameters that control the evolution of a given trait. The discussion examines how this model is affected by genotype, in order to select clonal material for commercial plantations. Some consequences for plant breeding strategy are also discussed.

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Title : APPLICATION OF NIRS TO THE PREDICTION AND THE ANALYSIS OF CHEMICAL WOOD COMPONENTS AND PAPER PROPERTIES

Abstract : Near infrared spectroscopy NIRS technique is now widely used for wood analysis. This paper presents some application in predicting the variability of the wood papermaking potential. The procedure, performed directly on wood sawdust, to obtain calibration models and their validation is first described. Then some predictions of chemical composition are exposed and discussed regarding wet chemistry determination. In the third part, the study focus on the pulping yield and some strength properties predictions of trees felt by the hurricane in France in december 1999. Finally some extended applications of NIRS predictions of papermaking potential about the selection of different genotypes and the following of the pulp properties of windthrown trees are presented.

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Title : LOCALISATION OF DIBENZODIOXOCIN STRUCTURE IN LIGNIFYING NORWAY SPRUCE XYLEM BY IMMUNOGOLD LABELING

Abstract : A polyclonal antibody made against an 8-membered dibenzodioxocin ring structure of lignin was used to study the lignification process in mature Norway spruce (Picea abies (L.) Karsten) xylem cell walls to reveal any difference in the distribution of this structure in the cell wall layers. Wood samples were collected in Southern Finland at the beginning of July, which is the time of active growth and lignification of the xylem cell walls. Wood blocks were fixed, cast in an acrylic resin (LRW) and sectioned for transmission electron microscopy (TEM). Sections of frozen material were also cut with a cryomicrotome for light and fluorescence microscopy. In very young cells where secondary cell wall layers were not yet formed, the presence of lignin, judged as the presence of the dibenzodioxocin structure, could not be shown. When the secondary cell wall layers began to develop, immunogold labeling showed the presence of the dibenzodioxocin structures in the middle lamellae. Later the 8-ring structure was detected in the primary and secondary cell wall layers. The number of the gold particles seen in the various cell wall compartments in the wood sections was counted. Statistically significant differences were found in the distribution of the gold particles. The results of the TEM immunogold labeling were compared with conventional safranin and alcian blue staining method of lignin and cellulose, respectively , in developing cell walls in Norway spruce xylem. Conventional staining methods and autofluoresence of lignin are not thought to be as specific in lignin localization as the TEM-immunogold techniques are. The distribution of the lignin benzodioxocin-structure was studied, because the many histochemical staining procedures (e.g. toluidine blue, safranin, fast green, pholoroglucinol) are insufficient since the specific reactions between the polymer and the dyes are not fully understood. Also, techniques based on autofluorescence of lignin substructures or application of fluorescent stains are hampered by fluorescence of non-lignin phenolics (non-polymerized monolignols) which are abundant especially in coniferous tissues. Lignin stains, autofluorescence of lignin and the immunogold technique were good tools to follow the advance of the lignification process, as seen in this study. TEM-immunogold technique made it possible to get information of the content of lignin in the developing cell wall layers in different developmental stages of wood tracheids. The 8-ring dibenzodioxocin lignin substructure was not described until 1995 in coniferous milled wood lignin by Karhunen et al. (1995a). The authors estimated that the biphenyl structure involved in this 8-membered ring comprises 19 to 26% of the phenylpropane units in softwood lignin, thus making it a very common structure in coniferous wood. The discovery of this 8-membered dibenzodioxocin ring structure may have important implications for understanding the chemical reactivity of lignin both in pulping and in biodegradation of wood (Karhunen et al. 1995b). The study presented here is the first to show where in the cell wall tissue this 8-ring structure is located.

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Title : DIFFERENT METHODS-DIFFERENT RESULTS: THE EFFECT OF FERTILISATION ON THE MICROFIBRIL ANGLE OF NORWAY SPRUCE AS SEEN BY X-RAY DIFFRACTION AND POLARISATION MICROSCOPY

Abstract : The purpose of this study was to determine the effects of growth rate on the mean microfibril angle (MFA) of Norway spruce (*Picea abies* (L.) Karst). Material for the study was sampled from a nutrient optimisation experiment in Flakaliden, northern Sweden (64°07 N, 19°27 E, alt. 310 m).At breast height, wood samples of 1cm*1cm*1mm were prepared from earlywood of every third annual ring from three fertilised and two control trees. Microfibril angle was measured by x-ray diffraction method using reflections 200 and 004[1]. The shape of the wood cells determined which reflection was used in analysis [2]. MFA distribution was presented as a sum of three Gaussian functions fitted on the measurement data. Parallel samples were macerated and MFA was also measured by polarisation microscopy [3].The x-ray measurements show an increase of 2°—5° in the value of MFA soon after the beginning of fertilisation. After the initial increase MFA remains slightly higher. When different sample trees are compared ring-by-ring, it is observed that the effect of fertilisation is smaller than the naturally occurring variation in the value of MFA between different trees, despite the increase in diameter growth caused by fertilisation. In the light of the results produced by x-ray diffraction the nutrient optimisation experiment has been very successful.The optical method produced higher MFA values than x-ray diffraction as a whole and the effect of fertilisation was also larger, making nutrient optimisation seem less useful. In order to understand why the results obtained by polarisation microscopy differ from x-ray diffraction a more thorough look on the physical properties of the tracheid and its components is needed.[1] Paakkari T, Serimaa R: A study of the structure of wood cells by x-ray diffraction. *Wood Sci. Technol. 18 (1984), 79-85*
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Title : WHICH WOOD PROPERTIES SHOULD BE SCREENED IN POPLAR BREEDING PROGRAMMES? A REVIEW.

Abstract : This review considers the influence of genetic and environmental factors on wood properties of poplar / aspen and their implications for selection and breeding programmes. Moderate to high levels of clonal variability are reported for many wood properties: basic density, tension wood content and growth stresses, tangential shrinkage, wet wood content, wood colour, cellulose and lignin content, fibre length and vessel proportion. Broad sense heritability of these properties is generally high, but the additive component of genetic variance appears to be rather small. Phenotypic and genetic correlations between growth and wood properties are rarely unfavourable. In most cases, clone ´ site interactions are not significant. Age - age correlations suggest that it should be possible to carry out early selection for wood properties when trees are one to four years old. Genetic variability is also found in technological properties of industrial importance: there are large differences between clones in modulus of elasticity, end splitting, wooliness, energy consumption and brightness of thermomechanical pulp, kraft pulp yield and paper properties. The prospects for selecting poplar (or aspen) clones with desirable technological / processing properties are good. Moreover, it appears possible to combine rapid growth with the wood properties required for different industrial processes. Presently, the emphasis in selection programmes is on basic density, tension wood content and wet wood content (for sawing and peeling), and cellulose / lignin ratio, wood colour and fibre morphology (for thermomechanical and kraft pulping). Further work is required to determine the relationships between basic wood properties and technological properties, and the optimum values of different wood properties for particular industrial requirements. Genetic improvement of wood properties would be better achieved by screening productive, pest resistant clones for those with the required properties than by a breeding programme. This is because of the absolute need for rust and pest resistance in commercial clones and the probable importance of non-additive effects in the genetic control of wood properties in poplar.

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Title : VARIATION OF WOOD PROPERTIES BETWEEN PLUS-TREE CLONES OF JAPANESE LARCH (LARIX KAEMPFERI CARRIERE).

Abstract : Japanese larch (Larix kaempferi Carriere) is the most important species to forestry in northern part and elevated land of Japan. Japanese larch can grow fast than other forestry species in those area, and wood density and strength in Japanese Larch is relatively higher than other coniferous species in Japan. Therefore, five handled and thirty plus trees had been selected, and two handled and thirty-two of them were selected at central highlands, which were Nagano prefecture, Yamanashi prefecture, and Gunma prefecture. However, there is only few data about wood properties of those plus tree clones. We tested spiral grain, annual ring components and modulus of elasticity of plus-tree clones selected at central district. Those are considered as good indices of wood quality in uses as solid lumbers. Broad sense heritabilities and expected genetic gains of those properties were estimated. We could get following results. *Spiral grain showed the highest genetic gain. *Modulus of elasticity and radial growth showed moderate genetic gains. *Annual ring components, which were considered as important factors in wood processing, showed the lowest genetic gains.

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Title : GENETIC AND ENVIRONMENTAL INTERACTIONS ON POPULUS SPECIFIC GRAVITY

Abstract : As part of a research program to develop superior clones of Populus to produce energy feedstocks, we are looking at genetic and plantation environment effects on specific gravity. An initial study of four promising clones showed specific gravity ranging from 0.304 to 0.362 in a planting managed under commercial cultural conditions. However, in a companion planting where protection against the cottonwood leaf beetle was not provided, stem specific gravity at breast height was reduced by up to 3.9% with a correlation coefficient of r = 0.95 between growth loss and reduction in specific gravity. A second study is assessing the impacts of spacing on specific gravity in the 'Crandon' clone of hybrid aspen. Typical patterns of vertical variation in specific gravity are seen in six-year-old trees, but the amount of variation along the stem is greater at closer spacings where the crown is being most heavily impacted by competition effects. A third study is focusing on variation in Populus deltoides families. A total of 78 selections were made for stem volume production across 36 families out of a beginning number of 42 families and 2594 seedlings. We expect this study to give us a much clearer test of the potentially negative relationship between stem volume growth rates and stem specific gravity that we have seen in studies with limited numbers of clones.

11
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Title : STIGMA DATABASE FOR TREE IMPROVEMENT PROGRAMS

Abstract : STIGMA is a Microsoft Access database developed to meet a need for integrated data management in the Shell Group Forestry Tree Breeding Programs and has been in use since 1998. The core of the application is the data associated with the resources seed, trees, clones and pollen. All subsequent use of the resource, via selection, pollen or seed collection, crossing, cloning or in trials is recorded. Linked data ensures that the pedigree of all trees, seed, pollen and clones can be traced back to the time of accession to the program or forward to the most recent generation of selections. The quantity and distribution of all germplasm to third parties and trials is also recorded. Activities (e.g. trials, clonal accessions) that carry commitments to share results or make conditional payments at some stage in the future are flagged in a Register of Agreements. Data output is in the form of query datasheets that are refined using selection criteria, filters and sorts or in fixed format reports. All data can be exported to MS Word and MS Excel. STIGMA is now the core for all tree improvement activities in Shell Forestry and has ensured the integrity of data over time or through staff changes. It will be available for commercial release from September 2001.

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Title : VARIATION OF MICROFIBRIL ANGLE IN SITKA SPRUCE (*PICEA SITCHENSIS* (BONG.) CARR.) FROM FOUR DIFFERENT PROVENANCES

Microfibril angle (MFA) represents the orientation of the cellulose crystallites in the cell wall along the fibre axis. Amongst other properties of wood cells the MFA determines the strength of individual wood cells or fibres and affects wood properties such as dimensional stability. The aim of the study was to examine the differences in microfibril angle in Sitka spruce trees from different provenances. Samples were taken from 13 trees that were grown in Ireland in similar environmental conditions. Seeds were collected from four different provenances in North-America: California, Oregon, Queen Charlotte Islands and Washington. Four earlywood samples from each tree were taken at breast height (1.3 m) in the stem from annual rings between 2 and 16. The MFAs of the samples were measured using X-ray diffractometer in symmetrical transmission mode (» = 1.541 Å). The reflections used were 200 at an angle of 2¸ = 22.4° and 004 at an angle of 34.6°. The expectation values of MFAs were calculated from Gaussian functions fitted to results. [1, 2] According to our results the MFA varied strongly not only as a function of the annual ring (or the distance from the pith) but also as a function of the seed origin (provenance). The MFA showed different trends from the pith to the bark in trees from different provenances and there was much less variation between trees within the same provenance. In the first growth rings, i.e. close to the tree pith, MFA varied from 15° to 32° and in the outer rings, i.e. close to the bark, from 6° to 23°. It is suggested that genetic differences between Sitka spruce trees from the four different provenances explain the remarkable differences in the general trends of the MFA behaviour.
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Title : GENETIC VARIATION OF WOOD DENSITY RELATED TRAITS AND THEIR RELATIONSHIP WITH GRADIAL GROWTH IN A RADIATA PINE PROGENY TEST

The genetic variation of wood density components (EWD: early wood density; LWD: late wood density; LWP: late wood proportion) and their relationship with weighted ring density (WRD) was assessed through cambial age in a radiata pine progeny test in the south of Chile. The genetic relationship of wood density with intraring density variation and radial growth was also studied. Wood disks were taken at breast height from 18-year-old trees of 31 open-pollinated families. Indirect-reading X-ray densitometry was used to determine wood density and radial growth related traits. Three patterns of variation in family mean WRD from ring to ring were detected, which explained the decreasing in heritability through cambial age. More family variation occurred durig the first five rings in WRD. Heritability for wood density components (EWD and LWD) followed a different ring to ring trend compared to WRD. Early wood showed more intraring density variation than late wood. EWD was also better correlated to WRD than LWD (family correlation > 0.7 for every ring). A negative correlation between late wood area and LWD was recorded for every ring. The cumulative proportion of latewood decreased from rings 2 to 5 and stabilized close to 30 % after ring 6.

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Title : WOOD AND BIOMASS PRODUCTION FROM A SHORT ROTATION EXPERIMENTAL PLANTING OF TEN POPLAR CLONES IN N. GREECE

In this work the results of diameter and total height growth, wood and biomass production at age of 3 years of ten poplar clones are presented. Clones were established in an experimental planting at the left bank of Strymonas River -N. Greece at 1.0 × 1.0 m spacing, using the standard method of hardwood cuttings. Poplar clones used in this study were the following: I-74/76, I-77/74, I-81/74, I-102/74, BELLOTO, TIEPOLO, CIMA, I-214, I-45/51 and He-X/3. The aim of this study was the production of woody biomass (above-ground) using a harvesting cycle of 3 years.

The results of the first cutting cycle (end of third growth period) demonstrated that the growth of diameter at breast height (DBH) and of the total height (H) were significantly differed among clones. From the analysis of variance components, broad sense heritabilities (h^2) for DBH and H were estimated. The production of fresh biomass (FW), dry biomass (DW) and the moisture content (mc) for the different clones were also calculated. Significant differences between clones were found in respect of the referred parameters. Interestingly, the widely and commonly used in poplar plantations (in Greece and Europe) clone I-214, could not compete (in biomass production) other tested clones in that narrow spacing. The growth of DBH proved to be highly correlated with biomass production (r^2 = 0.73 - 0,97, $P<0.001$) and clones with the highest diameter growth (e.g. I-81/74, He-X3) achieved the highest biomass yield. Furthermore, medium productive clones (e.g. I-77/74, BELLOTO, TIEPOLO, I-102/74) produced acceptable biomass quantities and can be used in mixed poplar plantations to increase biological diversity and stability and reduce possible insectious and fungal attacks.

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Title : MAPPING QTLS CONTROLLING ENZYME ACTIVITIES IN MARITIME PINE SUBMITTED TO OZONE STRESS

Abstract : At the present time, trees have to cope with climatic changes that will probably become more significant in the future. The previsions for next decades indicate that some atmospheric gases will drastically increase. The tropospheric ozone is one of these gases, its concentration over Europe increases continuously with peack concentrations up to 150 nL L-1 in spring and summer. Ozone stress causes decrease in tree growth by affecting physiological processes like photosynthesis, CO2 uptake, carbon allocation and partitioning to different components of the tree. In previous studies we showed that enzymes from different pathways were affected by ozone exposure. RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity decreased, however the PEPC (phosphoenolpyruvate carboxylase) activity increased. In the shikimic pathway, a link pathway between primary and secondary metabolism, the ShDH (shikimate dehydrogenase) activity increased and in the phenylpropanoid pathway a correlated increase in activities of PAL (phenylalanine amonia-lyase) and CAD (cinnamyl alcohol dehydrogenase) was observed. The correlation of the enzyme response led to ask the question: is there common genetic factors controlling the different enzyme activities in response to ozone? To answer this question we developed a QTL mapping strategy to localize the chromosomal regions accounting for part of the variation of enzyme activities. Eighty-one two year-old seedlings of a three generation inbred progeny of maritime pine were used. A saturated linkage map of the hybrid tree was available. One year-old needles were harvested for enzyme analysis before and after 27 days of ozone exposure (200 nL L-1). Thirteen QTLs were identified for the different enzyme activities in the non-stressed and ozone treated condition. Interestingly, a co-location between QTL for PAL activity (in both conditions) and the PAL gene was observed. An other important co-location between QTLs for ShDH and CAD activities under ozone exposure was found. These results emphasize the possible role of candidate structural genes in the control of enzyme activity and the existence of common regulatory genes involved in the control of enzyme activities of the primary and phenylpropanoid metabolism in response to ozone stress.

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Title : COMPARISON OF QUERCUS ROBUR L AND CASTANEA SATIVA MILL. GENETIC LINKAGE MAPS USING SSR MARKERS

Abstract : Genetic maps for oak (Quercus robur, 2n=2x=24) and chestnut (Castanea sativa, 2n=2x=24), were constructed using the two-way pseudo-testcross mapping strategy. For oak, the consensus map comprised 503 markers (168 AFLPs, 271 RAPDs, 46 SSR and 12 STS) in 12 linkage groups and provided 90% of genome coverage. For chestnut, the consensus map comprised 381 markers (311 RAPDs, 65 ISSRs, 5 isozymes) in 12 linkage groups and provided 70% of genome coverage. Oak SSRs were tested for cross amplification in chestnut. Overall, 30% of them amplified in this species, providing anchor points for comparative mapping in the Fagaceae, 5 homologous linkage groups were recognised so far. The availability of microsatellite primers specifically designed for chestnut will facilitate the search of homology among linkage groups. Alignment of both genetic maps will provide a useful information regarding the evolution of the two genomes, and a tool to compare the location of the same trait QTLs that are being mapped in both species.

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Title : CONTRIBUTION OF MARITIME PINE FOR COMPARATIVE MAPPING IN CONIFERS

Abstract : The sequencing of expressed genes (EST) and the development of Simple Sequence Repeats (SSR) provide orthologous markers which are useful for comparative genome mapping in conifers. If the ordering of genes is maintained, then these species can be considered as a single genetic system. At a more practical level, this would allow to transfer information (QTL, candidate genes) between species. This discipline could also allow studying the evolution of their genome. Several research organisations worldwide are developing primer sets to amplify EST and SSR that are used as anchor-points between the genetic maps of approximately ten conifer species belonging to the Pinaceae. In this study about 200 ESTs and 60 microsatellites were tested for amplification in Pinus pinaster. Some were mapped and their position was compared to the Pinus taeda reference map. It was shown that gene order was well conserved between both species. This report agrees with others pairwise pine genome comparison and shows that pine genomes did not evolve in term of chromosomic rearrangement since their divergence. This encouraging result should allow interspecific validation of QTL location and will ultimately contribute to the development of marker-assisted selection.

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Title : TWO LINKAGE MAPS OF WHITE SPRUCE (*Picea glauca*) BASED ON RAPD, ESTP, ESTP-SSCP AND SCAR MARKERS

Abstract : We have constructed linkage maps for two parents (M2 and 80132) of white spruce (*Picea glauca* (Moench) Voss). Haploid megagametophytes from 92 and 96 seeds of M2 and 80132, respectively, were analyzed with RAPD, ESTP and SCAR markers. Fragments segregating in a 1:1 Mendelian ratio were classified and mapped using MAPMAKER and JoinMap. For M2, the analysis with JoinMap resulted in 165 markers (153 RAPDs, 9 ESTPs and 3 SCARs) mapping to 23 linkage groups and covering 2059.4 cM(K). For 80132, the analysis resulted in 144 markers (136 RAPDs, 7 ESTPs and 1 SCAR) mapping to 19 linkage groups and covering 2007.7 cM(K). The maps covered 87 and 73% of the entire genome of parents M2 and 80132, respectively. Similar results were obtained with MAPMAKER. A comparison was made between the two maps, and 16 markers were shared between the two individuals.

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Title : DETECTION OF QUANTITATIVE TRAIT LOCI FOR AUTUMN COLD TOLERANCE IN *PINUS SYLVESTRIS* ACROSS YEARS

Abstract : Quantitative trait loci (QTLs) related to autumn cold tolerance in *Pinus sylvestris* were analysed with respect to their effects and locations on AFLP genetic linkage maps developed from both parents of a hybrid progeny. An evaluation of cold tolerance in 94 individuals of a full-sib family growing in a field trial in northern Sweden, which gave indications of individual variation in autumn frost hardening, was carried out in September 1997 and September/October 1999. Considering all the data, nine different QTLs were detected related to frost hardening, but the number of QTLs varied from two to five depending on the date of the trait evaluation. The percentage of phenotypic variation explained by a single QTL ranged from 8.7 to 28.2%. The high percentage observed in some cases suggested that major genes could be involved. None of the QTLs detected in September 1997 were significant in September 1999 and, conversely, some QTLs that were not detected in September 1997 were significantly expressed in September 1999. Some QTLs observed in September 1999 were also found to be significant in October 1999. The difference in expression at the gene level is discussed in relation to the climatic variations observed between the years 1997 and 1999, especially differences in temperature between the two freezing periods.

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Title : DETECTION OF QTLS ASSOCIATED WITH RESISTANCE TO TWISTING RUST IN MARITIME PINE

Abstract : A F2 progeny of maritime pine was assessed for resistance to twisting rust caused by Melampsora pinitorqua. This progeny was obtained from the self pollination of a single hybrid tree derived from a cross between trees from Corsican and Landes provenances, and previously used for the construction of a genetic map. The plant material was available as grafted samplings grown in a nursery, with one copy per individual. Artificial inoculations under controlled conditions were performed on excised shoots cut from this material at 3 dates during spring (i.e. the susceptibility period). A local wild inoculum of M.pinitorqua was used. The level of rust resistance was determined by several parameters related to the percentage and the severity of infection observed at different times after inoculation, in each of the 3 dates. Significant genotype and inoculation date effects were obtained for most parameters. Nine QTLs associated with rust resistance were detected. They were localised on 8 out of the 12 linkage groups of the map. QTLs were associated with the different resistance parameters, and each explained 10 to 25% of total phenotypic variation. However, putative QTLs were specific of the date of resistance assessment, i.e. none of the QTL detected at any one date was also detected at another inoculation date. In the same investigation, a major gene associated with shoot colour was also localised. Two co-localisations between putative QTLs for rust resistance and QTLs for cutting ability were observed. No co-localisation occurred with other previously detected QTLs or genes associated with growth, terpene synthesis, stress-related enzymatic activities or shoot colour.

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Title : GENETIC DETERMINISM OF LIGNIN CONTENT IN MARITIME PINE AND BREEDING IMPLICATIONS

Abstract : Maritime pine is the first conifer species used for reafforestation in France, where it covers 1.5 millions ha. Until now, despite its wood is widely used in pulp industry, only small attention was given to its chemical composition and especially to lignin, an undesirsable component in the pulp and paper industry. The objectives of this study were to determine the extent of genetic control and to study the genetic architecture of lignin content in this species. Lignin content was measured using mid-infra-red Fourier transformed spectroscopy in two experimental trials: (i) a 12 x 12 half-diallel to estimate the genetic parameters, and (ii) a three-generation outbred pedigree to detect QTLs. These two designs involved 16 year-old trees. Lignin content was found to be under fairly good genetic control, with a narrow sense heritability of 0.42. Despite a low phenotypical variation (cvP = 4 %), we will show that genetic gains, interesting at the industrial level, can be obtained. Using a saturated genetic linkage map based on AFLP markers genotyped on 200 trees, five putative QTLs, accounting for 31.7 % of the total phenotypic variation, were detected. These results open new possibilities to select lignin content based on the IR technique (a cheap, precise and high throughput method) or molecular markers. However, before marker-assisted early selection can be implemented in the breeding programme, the genes underlying the detected QTLs will have to be discovered.

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Title : GENETIC ARCHITECTURE OF TRAITS DIFFERENCIATING TWO EUROPEAN OAK SPECIES: QUERCUS ROBUR AND QUERCUS PETRAEA

Abstract : Q. petraea and Q. robur are two closely interfertile species that exhibit phenotypic differences but only show extremely low genetic differentiation for isozymes, chloroplast or nuclear DNA markers. Morphological characters (petiole length, hairiness and number of intercalary veins), are among the main features involved in species differences. In order to identify genomic regions involved in the molecular differentiation of the two species a statistical approach is conducted based on QTL detection for traits related to leaf morphology, and Ecophysiological traits (carbone isotope discrimination). Two maps were first established from the segregation of 500 markers (AFLP, RAPD, isozymes, microsatellites and STS) in a full-sib family of Quercus robur (278 offspring). The codominance and random distribution of 50 microsatellites, allows to define homology between linkage groups of both parental maps, and made it possible to construct a consensus map. QTL and candidate genes involved in the genetic differenciation are being mapped to investigate whether QTL/genes that differentiate both species are clustered on particular linkage groups or dispersed all over the genome.

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Title : TRANSGENIC SILVER BIRCH (BETULA PENDULA) CARRYING EITHER PTCOMT GENE OR PTCOMT PROMOTER-GUS CONSTRUCTS

Abstract : Introduction The target gene for the present study is a bispesific caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT) isolated from aspen (Populus tremuloides) together with its promoter [1,2]. The objectives were: 1) To introduce CaMV 35S or sunflower UbB1 promoter - PtOMT gene construct into silver birch and to study the content and chemical composition of lignin in plantlets. Hypothesizing that the homology between aspen COMT and endogenous birch COMT is not too high, COMT activity should be increased thus resulting in a higher amount of more easily degradable S units. In the opposite case, co-suppression may reduce or even silence COMT activity affecting both amount and solubility of lignin. 2) To introduce both full length and deleted PtCOMT promoter - b-glucuronidase (GUS) reporter gene constructs into silver birch and to study GUS expression in plantlets. In order to avoid potential malfunction of plant defence systems, it would be important to express modified lignin only in developing xylem. Material and methods Four in vitro birch (Betula pendula Roth) genotypes were used as targets, and transformations by particle bombardment, regeneration of transgenic plants, and Southern analysis using nptII, Ptcomt, and gus -specific probes were performed according to Valjakka et al. [3]. For studying GUS expression during different stages of growth and development as well as after wounding, histochemical techniques described by Nilsson et al. [4] and Chen et al. [5] were used. Current results Transgenic birches carrying different gene constructs have so far been produced and grown in the greenhouse experiments as follows: four full length PtCOMT promoter-GUS lines, five deleted PtCOMT promoter-GUS lines, four 35S-GUS lines, two 35S-PtCOMT lines, and three UbB1-PtCOMT lines. In addition, more lines have been produced but are still growing in vitro. According to Southern hybridisations the transgene copy numbers of the greenhouse-tested lines vary from one to nine. The growth and morphology of the plants have been normal, except in a few 35S-GUS and UbB1-PtCOMT lines showing dwarf phenotypes. For the lines carrying the PtCOMT gene, gene expression studies using RT-PCR and Northern technique as well as lignin content and structure analysis are currently under progress. Preliminary results, however, show changes in lignin S/G ratio of 35S-PtCOMT lines. In the lines with PtCOMT promoter-GUS reporter gene constructs, GUS expression patterns vary among regenerated genotypes. In the majority of the lines, the PtCOMT promoter activity is seen in vascular tissues of both stem and roots together with variable activity in leaves. Generally, more expression is observed when the reporter gene is driven by the deleted version than by the full length promoter. It is also obvious that the GUS activity changes according to season, being silenced in the hibernating plantlets. In spring, the PtCOMT promoter is first activated in phloem and/or in xylem ray cells. No additional PtCOMT promoter activity could be observed after mechanical wounding of either leaves or stems of the selected lines, suggesting that the promoter is not wound inducible.

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Title : INTER AND INTRA FAMILY EFFECTS ON REINITIATION AND INDUCTION OF SOMATIC EMBRYOGENESIS OF *Pinus maximartinezii* Rzedowski

Abstract : Somatic embryogenesis in pine species has been considered recalcitrant for many of these conifers tree. Initiation of embryogenic tissue is achieved in very low percent and even less establishment of embryogenic tissue. In spite of a considerable progress in somatic embryogenesis of other conifers tree, no study has ever been shown to produce high reinitiation from excised embryogenic masses produced into mature female gametophyte and high induction of somatic embryos from excised precotyledonary zygotic embryos or into female gametophyte and their establishment from each selected family of pine species. Our aim was to assess the family and individual genotype effects on *Pinus maximartinezii* over reinitiation, induction and their establishment of somatic embryos from each selected family. 4 families randomly selected were used for reinitiation and 5 families for induction of somatic embryos. The basal media utilized were modified with various ammonium to nitrate molar ratios, mixtures of plant growth regulators, mixtures of carbon source and other compounds as activated charcoal and gelling agents. As many as 20 genotypes were established out of 23 embryogenic masses belonging to 4 families. About the induction of somatic embryos and their establishment has been achieved from 95 genotypes out of 129 precotyledonary embryos belonging to 5 mother trees in a range from 50 to 93% of those zygotic embryos depending on family. There are differences among families over the production of embryogenic masses but no differences in reinitiation and establishment of proliferation. Over de induction on precotyledonary zygotic embryos, the mother trees shown differences in seed production but no significant differences on induction capacity and establishment of proliferation. The media compounds have been shown no effect on reinitiation and induction of somatic embryos in *Pinus maximartinezii*.

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Title : MICROGRAFTING OF WAVY GRAIN MAPLE

Abstract : The wood of wavy grain maple, a variety of sycamore maple (Acer pseudoplatanus), is one of the most expensive ones and used for veneer and music instruments. Nevertheless maple trees, especially selected older ones, are difficult to propagate by ordinary tissue culture techniques. A rapid callus formation, but no or only a limited organ formation under the influence of thidiazuron is often the result. Several possibilities of juvenile rootstocks for the application of micrografting techniques were tested. Shoot segments from a juvenile maple clone, germinating seedlings and hairy roots from a juvenile maple clone achieved by treating with a wildtype A. rhizogenes strain were used. Shoot segments showed a rapid formation of the graft union, nevertheless a later induction of roots led to a dying off of the grafted parts, because there was no phytohormone support during the root formation period. If the root was formed rapidly the grafted parts survived and started to sprout, thus a rooted shoot segment was found to support the grafting process best. Germinating seedlings in vitro formed a graft union as well but the graft union had to be fixed by a clip of silicon rubber. The lignified parts of hairy roots were found to be a suitable rootstock. Grafted meristems started to flush soon after grafting and showed stem axis elongation. This is the first time that such a kind of rootstock was used in maple and it offers a possibility for micropropagation. The material derived from micrografts will be observed to study effects of the different rootstocks on growth behaviour and performance concerning a possible rejuvenation, which would allow a common vegetative propagation by cuttings later. The material will be used as well to establish efficient micropropagation systems based on organogenesis. Moreover the collection and propagation of such a valuable material offers the possibility to study not only the expression of the wood structure after grafting, but offers at the same time a basis for later investigations about the heretability of this wood structure on a molecular basis.

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Title : MOLECULAR AND QUANTITATIVE TRAITS CONFORMITY OF EUROPEAN ELMS (ULMUS SP.) REGENERATED FROM LONG-TERM CRYOPRESERVED BUDS

Abstract : Elm (Ulmus sp.) is a greatly appreciated species in demand for high quality, strong and ornamental timber. Genetic ressources from the 3 indigenous European species (U. minor Mill., U. glabra Huds, and U. laevis Pall.) as well as from natural hybrids between U. minor and U. glabra are greatly endangered owing to recurrent Dutch elm disease during the last century, resulting in considerable reduction of adult elm populations. In France and Europe, in and ex situ conservation programs have been initiated in the past 15 years for the 3 species. However, it is largely unknown if such cutting-based programs in conjunction with natural vegetative (shoot, sucker) or sexual regeneration (seed) is sufficient to avoid genetic erosion of the elm germplasm. More recently, AFOCEL has developped alternative, long term and efficient ex situ techniques of organ or tissue conservation in liquid nitrogen (cryopreservation) for several conifers and angiosperms, especially Ulmus species. At present, dormant buds of 226 clones have been cryopreserved in the frame of the EU RESGEN conservation program (CT 078-96) of elm genetic ressources. Regeneration potential of dissected cryopreserved apices directly placed on culture medium is high for U. minor (39-100%), U. laevis (30-95%) and U. minor-glabra (52-73%). Interestingly, U. glabra apices only survived when micrografted on U. minor microcuttings (44-96% survival rate). Non cryopreserved (control cuttings) and cryopreserved material (cuttings and microcuttings) from 3 clones of U. minor and 3 clones of U. laevis were compared in field plantation test (randomized blocks of 5 plants) to assess quantitative traits and genetic conformity. Considering one phenological (flushing) and 5 morphological traits (height, collar diameter, number of branches longer than 10 cm, mean surface and length/width ratio of 3 leaves) after two vegetation years, no clear differences between cryopreserved and control plants could be detected. Significant differences observed for some clones were all in favor of cryopreserved plants. Molecular PCR investigation of genetic conformity were performed using 5 different primers (15-19 pb) designed to amplify variable number of tandem repeats (minisatellite regions). All clones could be distinguished using these markers. Similarity indices (0.92-1.00) based on presence/absence of fragment 500-2500 bp long with defined relative intensity strongly suggested molecular identity between cryopreserved and control plants.

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Title: STABLE TRANSFORMATION OF NORDMANNS FIR (*ABIES NORDMANNIANA*) BY PARTICLE BOMBARDMENT AND REGENERATION OF TRANSGENIC PLANTS.

Abstract: Nordmanns fir (Abies nordmanniana) is grown on a large scale in Denmark for the purpose of producing Christmas trees. The best provenances are slow growing, late flushing, with uniform growth and an ability to retain needles long after feeling. Propagation for Christmas tree plantations is exclusively by seeds, because cuttings are difficult to root and tend to grow plagiotropically. Due to the large economical importance of the species, there is a considerable interest in developing methods for vegetative propagation trough somatic embryogenesis and to combine these methods with introduction of specific traits through the use of genetic transformation. Recently we were successful in stable transformation of embryogenic *A. nordmanniana* cultures through particle bombardment. The inserted plasmid was pCW 122, which includes the reporter gene, uidA (GUS) and the selection gene, NPT II. The transformed cell lines have shown high embryogenic capacity and more than 1000 plants are presently growing for the second season in green houses. Thus, the present results show that it is possible to introduce new genes into embryogenic cultures of Nordmanns fir, and that large numbers of plants can be produced from these cell lines.

The next step in this work is to incorporate specific genes that will have practical importance for growers and thereby improves the value of the produced plants. The transgenic trees that have already been produced will be used for field tests and for evaluation of the ecological aspects of introducing transgenic plants in forestry.

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Title : INITIATION OF SOMATIC EMBRYOGENESIS FROM CONTROLLED CROSSES IN PINUS PINASTER AIT.

Abstract : Pinus pinaster is one of the most important species for the French wood and paper industries. It covers more than 1 million ha. Strong efforts are focused to genetically improve the species and make available more efficient sylviculture practices. Somatic embryogenesis is presently considered as part of the breeding programs. This paper presents a summary of AFOCEL results on initiation of somatic embryos clones from immature seeds since the early experiments in 1988 until 2000 with a focus on unpublished data (year after 1994). Immature embryos from controlled pollinated elite trees have been harvested during the summer time, taking into account the competence window defined in late 80's. The initiation rate of embryonal suspensor masses (ESM) was strictly scored at the level of stabilized proliferating material to avoid artificial overestimation. It was revealed to be highly variable both among crosses and among years for the same cross despite harvesting of plant material at the same time of the year and using only embryo at the precise developmental stage defined as optimal. Medium comparison during rapidly concluded to the superiority of DCR, which allows more regular and higher results on average despite not being the best in each particular case. In addition to precluding the checking of the developmental stage of each zygotic embryo (ZE) put in culture, presence of the megagametophyte tends to give us a lower initiation rate. No correlation between ZE development and temperature data could be observed, especially during the last years that were significantly warmer in the Landes area where are located the seed orchards. No strict parental effect could be seen but availability of cones of a particular cross is difficult to obtain regularly each year, making very hazardous the possibility to replicate experiments. The results merely suggested a physiological influence of the mother tree rather than a strictly mono- or bi-parental genetic effect. When weather and cone characteristics are good, initiation rates average 45%, reaching 75% for some crosses with the recovery of ESM from each family. Key words: Pinus pinaster, initiation, genetic improvement

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Title : PHOTOPERIODICAL CONTROL OF POST HARDENING GROWTH IN NORWAY SPRUCE SOMATIC EMBRYOS

Abstract : While somatic embryogenesis of spruce species is pretty well mastered by numerous labs, some teams still have difficulties to produce well growing plants. One of the factors explaining such a situation is the management of ex vitro growth and particularly the period of time just following the transfer of plants from the lab to the greenhouse. After having improved all classical parameters of greenhouse plant management, we still had troubles of precocious growth arrest of terminal buds. This blocking was irreversible in most of the case, leading to abnormal morphologies. The solution to this problem was found empirically in early the 90's at AFOCEL, as it seems to have been the case in many other labs at the same time or several years later. It consists in using extended photoperiod for some weeks until the terminal bud will have gone through the critical stage of growth restart after the stress of weaning. As the things worked fine, we don't assign a high priority to the proper scientific validation of this technical point. We are now presenting detailed experimental results on several clones showing precisely what is the impact of different light regime on the growth of young emblings along their first months outside the lab. Key words: Picea abies, light regime, plant quality

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Title : SUBCULTURE EFFECT IN NORWAY SPRUCE SOMATIC EMBRYOGENESIS.

Abstract : Somatic embryogenesis is quite well mastered for spruce species. At least in western Europe, the most limiting factors for it's practical use are the high manpower requirement leading to a production cost somewhat higher than that of standard seedlings and the lack of interest for clonal propagation (increased by the confusion between clone and GMO) of most foresters. We designed an experiment to situate the security limit to warrant reliable and efficient plant regeneration from somatic embryos culture in terms of subculture number or total time of continuous multiplication. A large amount of 4 different performing lines where cryopreserved after a limited number of subcultures while they where maintained in continuous culture. Cryovials where thawed along a 100 weeks period after increasing amount of time and plant material reactivated. At each of the 10 time points, stably reproliferating cultures where matured in parallel with unfrozen and, if already present, previously thawed ones. Except for one line showing a very fast degeneration, no long-term stable trend could be detected at the level of plant regeneration ability. Plant production was either increasing with subculture number for sometimes quite long period (40 weeks for example). If maturation yield varied significantly according to the multiplication and maturation systems (solid or liquid culture, spreading or not ESM in thin layer for maturation...), the evolution of mature embryo numbers where quite parallel. The line showing a very fast collapse of maturation ability (down to almost zero in 3 months) had a microscopical aspect typical of B lines (after the terminology of the team of S. von Arnold) concomitant with a granular aspect with fast draining and drying of ESM sedimented from suspension culture. The best results were always obtained with material matured as a thin layer on filter paper laid on the top of solid medium from a proliferation done either on solid medium or in suspension. At the level of sanitary safety (susceptibility towards infection) as well as at the level of cost of long term maintenance of a minimal amount of plant material, the solid proliferation prove to be the best and the cheapest. In conclusion, we choose to adapt the time of continuous maintenance in culture to the speed of the maturation ability lost. Each newly initiated line is cryopreserved as soon as possible and then its the relative speed to loose the potential of plant production is estimated on maturation test replicated at least 5 times along several months. The time limit is thus know for fast decaying material while more persistent lines are not maintained for more than one year but this limit was chosen considering sanitary risks overall. Key words: Picea abies, culture cycle, somatic embryos, maturation

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Title : SOMATIC EMBRYOGENESIS IN PINUS PINASTER AIT.

Abstract : There are more than 4 million hectares of Pinus pinaster forest in mediterranean Europe. Among these countries, somatic embryogenesis of this species has been studied for years mainly in France (AFOCEL and INRA), while some projects later in other countries. Australia is also becoming an important pole with the long term objective to reach at least 500,000 ha. They started also to work on somatic embryogenesis a few years ago. In France, while work started in late heighties, some difficulties still persist. At the starting point of the process, the initiation rate from controlled cross immature seeds is high and proliferation is efficient, leading to a high rate of stabilized immature embryos, proliferating in colonies called embryonal-suspensor masses (ESMs). Cryopreservation is routinely done with a high success, leading to large cryobank establishment allowing to secure a significant part of the genetic variability of breeding populations, allowing to integrate somatic embryogenesis in breeding programs like most of the big forest companies are now very actively doing with their species of interest. Like with other conifers, ABA is not sufficient to trigger the differentiation of immature into cotyledonary embryos (stage 3). The development of immature embryos into cotyledonary embryos is one of the major problems preventing the large deployment of somatic embryogenesis in Pinus pinaster. But significant progress were made during the last years. The influence on the maturation of maritime pine somatic embryos of carbon sources and PEG combined with increasing concentration of gellan gum was tested. The effect of the 2 factors vary widely between lines. Some lines showed a striking response to PEG treatment, which limited the ESM proliferation and enhanced the maturation rate. Conversely, proliferation was stimulated by PEG in other lines without subsequent improvement of the maturation rate. The use of high concentration of gellan gum (0.9%) improved the maturation of all ESM lines. It was concluded that the more efficient culture medium to recover cotyledonary embryos from all lines was supplemented with sucrose at 6% (w/v) and gellan gum at 0.9% (w/v) without PEG. It apperas also that the maturation performance is highly influenced by sampling modalities; the outer part of the ESM yielded more cotyledonary embryos than the inner part or the whole colony. ESM lines showing several stage 1 embryos at the periphery (spiky) were more productive than those for which stage 1 embryos were rarely visible (smooth). Impact of gelling and carbohydrate on plant regeneration will be also discussed together with the effect of genetic component. Key words: Pinus pinaster, breeding, somatic embryos, plant regeneration

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Title : EFFECTS OF THIDIAZURON AND HORMONAL STARVATION ON JACK PINE MERISTEMATIC NODULES

Abstract : Jack pine (*Pinus banksiana* Lamb.) is a coniferous species with a high potential for reforestation and there is great interest for the clonal mass production of superior genotypes. We therefore attempt to establish an efficient tissue culture system, based upon meristematic nodules. Nodules were induced from zygotic embryos in liquid half-strength SH basal medium (Schenk, R.V. and A.C. Hildebrandt 1972. Can.J.Bot. 50 : 199-204) supplemented with benzylaminopurine and kinetin and were subsequently cultured on membrane rafts floating on the same basal medium. The effects of hormonal stress were investigated by applying a pulse treatment of thidiazuron, a highly potent cytokinin, followed by culture on hormone-free media for 12 weeks. Different pulse durations (3, 6 and 9 days) and concentrations (0, 0.01, 0.1 and 1 m M) were tested and compared for their effect on organogenetic potential (bud and shoot production) and genotypic survival rate. For all treatments, bud production could be observed three weeks after the pulse and shoots were visible after nine weeks. The duration of the pulse influenced bud production, the three-day treatments being more productive. For shoot production and genotypic survival, the concentration of thidiazuron had more effect, the control (0 m M) and the lowest concentration (0.01 m M) showed slightly better results. Preliminary experiments with activated charcoal (5 g/l) and indol-butyric acid (2.5 m M) failed to promote the axis elongation of isolated shoots after three months on solid media. The effect of a 6-week culture on thidiazuron (0.001 and 0.0005 m M) and hormone-free media was also investigated on meristematic nodules which had been maintained in multiplication phase for over 2 years. The treatments enhanced bud production but caused pronounced browning. These results will contribute to a better understanding of the hormonal requirement of jack pine in vitro and to the further development of the micropropagation system.

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Title : EFFECTS OF NITROGEN ON THE CELL CYCLE, NUCLEAR DNA AMOUNT AND NITRATE REDUCTASE ACTIVITY OF JACK PINE CELL SUSPENSIONS

Abstract : Nitrogen appears to be the most important nutritive element for plant growth both *in vivo* and *in vitro*. In this study we examined the influence of various nitrogen sources on the cell cycle, nuclear DNA amount, expression of $p34^{cdc2}$ protein (a cell cycle key regulator) and nitrate reductase (NR) activity of jack pine (*Pinus banksiana* Lamb.) cell suspensions. These were initiated in 1/2 Litvay (Litvay *et al.* 1981. Institute of Paper Chemistry Tech. Pap. Ser. 115: 1-17) basal medium including 50 mM NAA. In terms of nitrogen, the medium contained KNO_3 (950 mg/l), NH_4NO_3 (825 mg/l) and was supplemented with glutamine (438 mg/l). Quantitative DNA variations were first investigated, using flow cytometry analysis. Cell suspensions that were N-deprived over a period of 72, 84 and 96 hours showed a reduction in their nuclear DNA content in comparison to cells grown in 1/2 Litvay medium. The relative distribution of nuclei in the cell cycle phases (G0/G1, S, G2) varied between treatments. Cell suspensions were then grown either 1) in 1/2 Litvay medium, 2) in modified 1/2 Litvay without any nitrogen, or 3) were first N-deprived for 96 hours and then supplemented with various nitrogen sources (KNO_3 only, NH_4NO_3 only or glutamine only). For the different analyses, cells were sampled every 24 hours during 4 days. In suspensions continuously deprived of nitrogen, DNA amount decreased considerably as starvation duration increased, and the percentage of nuclei in the G1 and S phases were respectively higher and lower than in the control (1/2 Litvay). A similar change in nuclei distribution was observed with the medium containing only glutamine, without however a significant variation in the absolute DNA content. NR showed a greater activity when cells were cultured with NH_4NO_3 as the sole source of nitrogen. Western blot analysis of $p34^{cdc2}$ expression revealed a maximal variation of 1.9 fold throughout the treatments.

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Title : SOMATIC EMBRYOGENESIS AND AGROBACTERIUM-TUMEFACIENS MEDIATED GENETIC TRANSFORMATION OF ABIES NORDMANNIANA

Abstract : In Nordmann's fir, all steps of propagation via somatic embryogenesis (SE) and Agrobacterium-mediated gene transfer have been studied. SE was induced from mature zygotic embryos on DCR medium containing 10-30 µM BAP or 5 µM TDZ. Induction frequencies higher than 50% were obtained. Proliferation of embryogenic cultures (ESM) occurred on solid SH or in liquid DCR medium supplemented with 7.5 µM BAP. For embryo maturation, six basic media were compared: DCR, ½ MS, ½ BLG, SH, MSG and BMG 2. The best results were obtained on ½ BLG containing 40 µM ABA, 5 µM L-Glutamine, 0.38 mM L-asparagine, 7.5% (w/v) PEG-4000 and 3% maltose. For conversion embryos were cultured in light (16 h/d) on hormone-free BMG 2 medium. Rooted plantlets were subcultured on a substrate consisting of vermiculite and clay beads (1:1) soaked with ½ BMG 2 solution. After cold treatment at 5°C for 2-4 months to break bud dormancy, plantlets were established in soil. For transformation studies, A. tumefaciens strain GV 3101 was used, which contained the plasmids pPCV 812 or 814 and carried the reporter genes gus or gfp, respectively, and the marker gene hpt. Agrobacteria and ESM were co-cultivated for 2 days on DCR medium supplemented with 100 µM acetosyringone. Selection of transformants occurred on media with 2,5 - 10 mg/l hygromycin. At all concentrations, hygromycin was strongly selective and completely inhibited the growth of non-transformed ESM. Molecular and gene expression analyses of putative transformants, which grow vigorously on selective media, are in progress.

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Title : MARITIME PINE *PINUS PINASTER*: INDUCTION OF SOMATIC EMBRYOGENESIS FROM ZYGOTIC EMBRYOS COLLECTED FROM ELITE TREES

Abstract : An integrated pine-breeding program associating biotechnology with traditional breeding has been recently implemented in Portugal. This program is genetically based on a set of plus-trees selected in 1964-1966 from Leiria forest (Portugal). Somatic embryogenesis offers several advantages in improving forest trees (Jain, 1999) when compared to other in vitro methods. However, the use of somatic embryogenesis in improvement programs depends on the capacity to apply this methodology to a broad range of genotypes. In this work, our main objective was to evaluate the embryogenic ability of immature zygotic embryos collected from a representative sample of selected elite trees (Leiria provenance) that are being used for breeding purposes. One year-old open-pollinated female cones of *Pinus pinaster* were used in these experiments. The cones were collected in 1999 and 2000, from mother trees in Mata do Escaroupim, Leiria, Portugal. In 1999, the cones were collected twice a week between May 5th and July 30th, from 10-12 randomly chosen trees. In 2000, the cones were collected between June 30th and July 14th, from 20 clones of elite pine trees established in a clonal bank. Several basal media including DCR (Gupta & Durzan, 1985), LM (Litvay, 1985), BK (basal salts of Boulay, 1979 with MSG vitamins of Klimaszweska, 1989) and BM (Gupta & Durzan, 1985), supplemented with several concentrations of 2,4-D and BAP were tested. The DCR medium with higher hormonal concentration and supplemented with L-glutamine and casein was the tested condition that originated a higher number of embryogenic lines. When using the same medium formulation and increasing concentrations of 2,4-D and BAP, a decrease in the total number of induced embryogenic lines was observed. In year 2000, 1360 embryogenic lines were induced from 8854 explants inoculated (about 100-130 immature zygotic embryos were inoculated per induction medium and per family). The number of embryogenic lines induced per family varied from 13 to 195. Some of the induced embyogenic masses did not proliferate and after the fifth subculture some of the masses were eliminated. From these assays 1052 embryogenic lines were established. Maturation and cryopreservation assays are being performed based on previous work.

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Title : MOLECULAR ANALYSIS OF POTASSIUM DEPENDENT WOOD FORMATION IN POPULUS

Abstract : Increased vacuolation during cambial reactivation is correlated with changes in potassium content. We measured the concentration and distribution of potassium in poplar twigs, showing a key role of this ion in the generation of cambial turgor pressure. In addition, the distribution of the plasma membrane H+-ATPase which might be involved in the uptake of potassium into enlarging cells was studied by immunolocalization. In winter, no evidence for the enzyme could be found in sections of dormant twigs, but during cambial reactivation the enzyme was found localized in almost all cells of the cambial zone. Since the auxin content is high during cambial activation, we also proved the effect of auxin on the expression of the enzyme. These results suggest a role for the PM H+-ATPase in the control of cambial activity and the regulation of vascular cell enlargement. Furthermore, based on RACE techniques we screened a Populus EST library for ion channels and transporters. In an initial attempt we succeeded in the isolation of five different members of plant ion channels as well as two potassium transporters and several pumps. We cloned PTORK and PTK2 and started with their electrophysiological characterization using patch-clamp as well as impalement techniques.

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Title : MOLECULAR, CHEMICAL AND PHYSICAL CHARACTERIZATION OF TENSION WOOD IN EUCALYPTUS

Abstract : Tension wood (TW) represents for the tree an efficient mechanism allowing adaptation and harmonious development in its own environment. It differs from normal wood in terms of anatomical structure and chemical composition. These differences modify its physical properties and decrease its value as timber. We hypothesize that the formation of TW follows the same developmental program as normal wood, but that differentially expressed genes/proteins control the shape of the tracheids and fibers, the structure and the composition of their secondary cell wall. Differentiating xylem was harvested from a crooked Eucalyptus gunii adult tree. Protein extracted from different samples were revealed by high-resolution silver stained 2D PAGE and analyzed with a computer-assisted system for single spot quantification. Growth strain (GS) measurements allowed xylem samples to be classified quantitatively from TW to normal wood. Regression of lignin, cellulose and hemicellulose content on GS showed that a decrease in % lignin and % hemicellulose and an increase of % cellulose corresponded to decreasing GS values, i.e. TW. These results were confirmed on other eucalyptus species. Out of the 140 studied protein spots, 12 were significantly associated with GS: 7 being less expressed in TW and 5 being more expressed in TW. A clustered-correlation analysis was performed to study simultaneously protein expression along the gradient of gravistimulated stressed xylem tissue. Proteins were found to formed "expression clusters".

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Title : MOLECULAR CHARACTERIZATION OF A RECEPTOR-LIKE PROTEIN KINASE IN CONIFERS

Abstract : We are developing molecular approaches to study the growth and development of woody plants. As part of the research efforts carried out in our laboratory, we report here the molecular cloning and preliminar characterization of a cDNA from the conifer Scots pine (Pinus sylvestris), PinRP, encoding a polypeptide which is homologous to the receptor protein kinases described in angiosperms. A full-length clone was isolated from a cDNA library constructed from poly (A)+ enriched RNA prepared from germinating pine seeds. Characterization of the isolated sequence revealed that it contains a signal peptide involved in membrane targeting and multiple leucine rich repeats in the N-terminal region whereas a characteristic domain of Ser/Thr protein kinases is present in the C- terminal region. These conserved domains are separated by a putative membrane spanning sequence. RT-PCR analysis of PinRP transcript abundance in pine tissues suggest that the gene is expressed in embryogenic stages and developing seedlings. The overexpression of the protein kinase domain in E. coli is underway. Production of the recombinant protein will permit the generation of monospecific polyclonal antibodies and to characterize the functional properties of the enzyme.

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Title : TRANSGENIC POPLAR CHARACTERIZED BY ECTOPIC EXPRESSION OF A PINE CYTOSOLIC GLUTAMINE SYNTHETASE GENE EXHIBIT ENHANCED TOLERANCE TO WATER STRESS

Abstract : We previously reported that ectopic expression of a pine cytosolic glutamine synthetase (GS1) gene in transgenic poplar resulted in significant alterations of biochemistry, early growth and development of transgenic poplar. Analysis of young leaves 22 independent transgenic lines indicated that GS activity, protein and chlorophyll content on a fresh weight basis were respectively 65.8, 33.4 and 21% higher in the transgenic than in control plants. A linear correlation between GS expression and height was observed. These results suggest that GS activity in young leaves could be used as a marker of the vegetative development in the poplar lines. We also investigated the consequences of ectopic expression of pine GS1 on physiological responses hybrid poplar to water stress. Prior to withholding water for eight days, control plants showed higher net photosynthetic rates (Anet) under saturating light conditions, however transgenic plants had markedly higher levels of optimal photochemical efficiency of PSII (Fv/Fm) compared to non-transformed controls. In addition, light response dynamics of photochemical quenching (qP), and antennae-based thermal dissipation (NPQ) were similar between transgenics and controls at this time, while PSII antennae transfer efficiency (Fv'/Fm') and light-adapted PSII yield (fPSII) were consistently higher in transgenic plants. At full stress (soil water potentials = -3 MPa) Anet were markedly higher in transgenics compared to controls, and after one day of recovery, Anet were 77% higher in transgenics compared to controls. Stomatal conductances (gs) did not significantly differ between transgenic and control plants at any time. During water stress and 24 h after recovery, Fv/Fm were identical between the different plants, but after 3 days recovery, Fv/Fm in transgenic plants reached pre-stress levels, while levels in control plants remained low. Fully-stressed transgenics had 20% higher qP compared to controls, and upon recovery qP remained higher in transgenic plants, especially at lower light intensities. Fv'/Fm' was similar under full-stress for transgenics and controls; however, after 24 hrs and 3 days of recovery, Fv'/Fm' in transgenics was 7% to 20% higher compared to control plants. Higher qP and Fv'/Fm' accompanied significantly greater fPSII in transgenic plants during the full-stress and recovery periods. These findings suggest that overexpression of pine cytosolic GS1 resulted in an increased ability to sustain photosynthetic electron transport capacity during water-limited conditions. It is possible, therefore, that ectopic expression of cytosolic GS1 increases photorespiratory activity in transgenic poplar, and that this serves as an energy sink to better protect photosynthetic light-harvesting capacity.

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Title : A CANDIDATE GENE FOR MARKING RADIAL GROWTH OF HYBRID WALNUT: STUDY OF THE CDC2A GENE STRUCTURE

Abstract : Timber production is the main economic role assigned to forests. In order to improve the management of this resource, one of research aims is the understanding of the mechanism involved in tree radial growth. The research, here, was conducted on hybrid walnut (Juglans nigra x J. regia) and was organized to gain knowledge about the cdc2a candidate gene that is involved in cambial activity. First, the complementary DNA sequence of this gene was determined. The full-length cDNA sequence is 1217 base pairs. Second, the gene structure was studied: five loci were determined with at least 7 introns, one of them was entirely sequenced. A polymorphism based on intron length is located at 2 loci. The partial sequenced introns underline sequence polymorphisms at 4 loci. An evalutation of the copy number of cdc2a gene in hybrid walnut genome was also established and leads to an estimation between 3 and 6.

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Title : GENETIC TRANSFORMATION OF NORWAY SPRUCE (PICEA ABIES): OPTIMISATION OF BIOLISTIC METHOD ON EMBRYOGENIC TISSUES AND EVALUATION OF GUS AND ROLC GENES EXPRESSION IN TRANSGENIC LINES.

Abstract : Genetic engineering constitutes a new and powerful tool that can be used to understand fundamental processes in development and reproduction of trees, and also to produce trees which flower earlier, or alternatively, to produce sterile trees. Moreover as reproduction occurs relatively late in many tree species, the induction of early flowering might accelerate genetic improvement programmes. Towards the control of flowering, several genes have been isolated from different plant species. These includes homeotic genes such as MADS-box and particularly meristem-identy-genes. These genes involved in developmental switch from vegetative to inflorescence meristems are of great interest and were isolated for example from Arabidopsis thaliana, Anthirrinum majus and also from woody species such as Eucalyptus and Pinus radiata. Another kind of gene, the rolC oncogene of Agrobacterium rhizogenes is also interesting to understand development of trees. In fact, this gene is implicated in a “hairy root syndrome” characterised by several phenotypic modifications in the infected plant such as a root surproduction, a lost of apical dominance, a shorter inter-nodes, wrinkled leaves and a decreased production of pollen and seeds (1). Moreover in certain cases, this oncogene is able to induced early flowering (2) (3). Our present aim objective is to produce transgenic Picea abies (Norway spruce) plants which flower early. Therefore, a particle bombardment procedure was developed to genetically transform embryogenic tissues of Norway spruce. The introduced DNA construct contained the gus reporter gene and the rolC gene of Agrobacterium rhizogenes both under the control of CaMV 35S promoter and the nptII selectable marker controlled by the nos promoter. Firstly, we found that pretreatment of cells with high osmotic potential (0.25 M mannitol) enhanced gus transient expression. Secondly, scaling of others factors such as the particle size has been done to improve both the transient and stable transformation efficiency. Our results revealed that the level of transient expression didn’t reflect exactly the efficiency of stable transformation, as it was suggested before. Finally, the established method allowed the obtaining of 55 transgenic lines (6.2 transgenic lines for 100 shoots). The integration of transgenes into the genome of Picea abies was confirmed by PCR analysis. Over 80 % of these lines retained their embryogenic potential. Twelve transgenic lines were analysed and all contained the gene gus and rolC but only 7 shown an expression of the reporter gene and 11 of rolC gene with variable levels. And of these 12 transgenic lines, one line contained 2 insertions of transgenes, four lines contained 4 insertions and in the other, higher level of insertions (5-10) have been detected. Moreover, histochemical assays revealed that the majority of transgenic lines were chimeric and fluorometric assays indicated a low expression of gus gene in embryogenic masses. At this time, around 50 plantlets has been regenerated and acclimated but no phenotypic alteration of development have been observed for the instance. (1) Tepfer, 1984. Cell 37 : 959-967; (2) Kurioka et al., 1992. Plant Cell Rep. 12 : 1-6 ; (3) Giovannini et al., 1999. In Vitro Cell. Dev. Biol.-Plant 35 : 70-75

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Title : ETHYLENE AND IAA INVOLVMENT IN TENSION-WOOD FORMATION IN ASPEN TREES

Abstract : When stems or branches of hardwood trees are removed from their original position in space, i.e. bent or leaned, they form tension wood on their upper side. Tension wood fibres are rich in cellulose and consequently have low lignin content. Moreover, wood production is increased on the tension wood side. It is therefore of great interest to understand the molecular control of tension-wood formation. The plant hormones indole-3-acetic acid (IAA) and ethylene are considered as important regulators in tension-wood formation. IAA stimulates cell division and cell expansion in cambial tissues. Ethylene acts as a stress hormone in plants and increased synthesis has been observed at mechanical stress and tension wood formation. Using GC-MS and a micro-technique for IAA analysis the radial distribution of IAA across the cambial region tissues in tension wood and opposite side wood was visualised. A technique for analysis of the ethylene precursor ACC has been developed and used to study the ACC distribution in similar tissues. The changes in levels of IAA, ACC and ACC-oxidase, the enzyme that converts ACC to ethylene, were studied at different time points between 30 minutes and 26 days in trees induced to form tension wood.

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Title : FUNCTIONAL CHARACTERIZATION OF MYB TRANSCRIPTION FACTORS EXPRESSED IN XYLEM OF EUCALYPTUS

Abstract : The functional analysis of the Eucalyptus cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD) promoters in two heterologous systems: tobacco and poplar, have demonstrated that these genes are preferentially expressed in vascular tissues undergoing active lignification. Analysis of promoters deletions together with gel shift assays revealed that the regions involved in the vascular expression of the CCR [1] and the CAD [Rech, this congress] genes contain cis-elements corresponding to the Myb binding consensus motif [2]. These data suggest that MYB factors are potentially involved in the transcriptional regulation of CCR and CAD genes. In order to test this hypothesis, a Eucalyptus xylem cDNA library was screened using a degenerated oligonucleotide corresponding to a highly conserved motif in the DNA binding domain of Myb factors. Three distinct MYBs factors (EgMYB1, EgMYB2 and EgMYB4) were obtained. The analysis of the amino acid composition of EgMyb 1 and 2 DNA binding domains, suggests that they preferentially bind to MGSIIG motives as defined by [2]. Gel-shift assays performed with the recombinant EgMYB1 protein confirmed that this factor specifically recognized the MBSIIG site present than the CCR promoter. The effects of the Myb factors on the transcriptional activity of the CCR and CAD genes is studied via co-transfection experiments in tobacco plants and BY2 cells. Preliminary results indicate a different regulatory activity for each MYB studied. In order to decipher in planta, the role of the three Eucalyptus Myb factors, tobacco and Arabidopsis plants were transformed with constructs allowing either over-expression or down-regulation of each of the three Mybs. For down regulation, dominant negative mutation constructs were generated, i.e. only the DNA binding domains were placed under the control a constitutive promoter. Molecular, histological and biochemical analysis in the regenerated plants are in progress and results will be discussed. 1-Lacombe et al., 2000. Plant J. 23:663. 2-Romero et al., Plant J.14:273.

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Title : TOWARDS THE DEVELOPMENT OF INSECT RESISTANT PINUS RADIATA

Abstract : Insects are responsible for substantial losses in forest productivity in many parts of the world. Engineered insect resistance in trees should contribute value to both tree improvement and pest management programs. One of the strategies for enhancing insect resistance include insertion of one of the various endotoxin genes from the bacterium Bacillus thuringiensis . Using Biolistics®, a vector with the cry1Ac gene, which confers resistance to members of the insect order Lepidoptera, was co-bombarded with a vector containing the nptII selection gene and/or the uidA reporter gene, into radiata pine embryogenic tissue. Twenty one transgenic lines (transclones) from 6 genotypes were selected and plants were regenerated from several of the transclones. Transformation was confirmed by PCR amplification of the nptII and cry1Ac genes and by nptII and cry1Ac ELISA assay. The co-transformation efficiency for two unlinked genes was 95%. Gene expression studies have yet to be carried out to determine the expression levels of the endotoxin gene in the transgenic plants. Once levels have been determined, these plants will be tested in insect feeding trials.

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Title : AGROBACTERIUM-MEDIATED TRANSFORMATION OF PINUS RADIATA EMBRYOGENIC TISSUE

Abstract : Somatic embryogenesis is a propagation technology capable of producing many genetically identical (clonal) plantlets from a single seed of an elite family. A protocol using Agrobacterium tumefaciens to transfer foreign genes into Pinus radiata embryogenic tissue was developed. Co-cultivation parameters were improved to achieve reliable transient expression of the uidA reporter gene in embryogenic cells. After co-cultivation, tissue was successfully decontaminated, monitored for recovery and transferred to the selective agent geneticin. Recovery of stably transformed tissue depended upon various factors such as health, timing and concentration of selection agent. The presence of foreign genes in 25 transformed Pinus radiata tissue has been confirmed using nptII ELISA assay and uidA and nptII PCR. Embryos have been regenerated from some of the stably transformed embryogenic tissue and young germinants planted in soil.

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Title : CLONING AND CHARACTERIZATION OF THE PUTATIVE CYTOKININ RECEPTORS IN SILVER BIRCH *BETULA PENDULA*

Abstract : Cytokinins are a group of plant hormones, which have a central role in various processes involved in plant growth and development. The most prominent effects of cytokinins are linked to cell proliferation, bud and shoot formation, and leaf senescence. We have shown that a mutation in a receptor domain of a two-component signal transducer WOODEN LEG affects vascular patterning in the root of *Arabidopsis thaliana* (Mähönen et al. 2000: Genes Dev 14:2938-2943). A recessive mutation in the *WOL* locus results in reduced cell number and loss of phloem development. Recently, Inoue et al. (Nature 409:1060-1063, 2001) showed that CRE1/WOL is a true cytokinin receptor. Since the defect in cytokinin signaling causes a loss of specific cambial like cell divisions around the developing xylem in *Arabidopsis* root, we want to investigate whether *WOL*-like receptors likewise regulate cell proliferation in the vascular cambium of a tree. We have identified three *WOL*-like genes expressed in the cambial zone or the root tip of silver birch. Isolated fragments show high homology to the three CRE1/WOL-like histidine kinases characterized from *Arabidopsis*. Organ specific expression profiles will be determined. Functionality of the novel birch genes will be tested by their ability to complement the *Arabidopsis wol* mutation and by modifying the expression of the *CRE1/WOL*-like genes in transgenic birch trees. The project aims to address the genetic regulation of cambium activity.

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Title : POTENTIAL APPROACHES TO THE CONTROL OF WOOD QUALITY BY MANIPULATION OF CYTOSKELETON

Abstract : During differentiation of cambial cells into secondary xylem cells (wood formation), newly deposited cellulose microfibrils on the innermost surface of cell walls change their orientation progressively. The orientation of cellulose microfibrils in the secondary wall, referred to as the microfibril angle, is one of the most important characteristics that determine the properties of wood. In particular, the angle in the thickest middle layer of secondary wall is closely related to mechanical properties. Therefore, the control of orientation of cellulose microfibrils allows us to change the quality of wood and its products. Observations in woody plants have revealed that there is a close relationship between the orientation of cortical microtubules, which are one component of the cytoskeleton, and the orientation of newly deposited cellulose microfibrils in differentiating secondary xylem cells. Such a close relationship has been found in both normal and reaction wood. In addition, the localized appearance or disappearance of cortical microtubules is related to the localized deposition of cellulose microfibrils in secondary xylem cells, namely the formation of pits, spiral thickenings and perforations. These evidences indicate that cortical microtubules play important roles in the control of the orientation and localization of cellulose microfibrils in secondary xylem cells. Therefore, biotechnological control of orientation and localization of cellulose microfibrils in the cell walls by manipulation of cortical microtubules provides new tools to improve the quality of wood and its products.

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Title : LIGNIN FORMING ENZYMES IN NORWAY SPRUCE TISSUE CULTURE: CHARACTERISATION OF PEROXIDASES AND OXIDASES AND THEIR GENES

Abstract : We are utilising a *Picea abies* suspension culture (A3/85) that is able to form extracellular, native-like lignin (1, 2, 3), in order to identify the peroxidase and oxidase isoenzyme(s) and their genes, that are responsible for lignin polymerisation. The focus has been on the enzymes secreted into the culture medium which is collected on the fourth day after initiation of the suspension culture when the lignin formation is visible and the peroxidase activity is highest. The nutrient medium contains several peroxidase isoenzymes (acidic, slightly basic and highly basic groups) that are different from the cellular forms constituting 5 % of the total coniferyl alcohol peroxidase activity. Only one laccase-like isoenzyme is found in the culture medium. Peroxidases have been purified in order to get specific sequence data for the design of primers for PCR amplification of the lignin synthesising peroxidase genes. This far four peroxidases have been purified and sequenced from the N-terminus, but only one of them yielded sequence, the others being blocked. Cloning of the gene fragment is in progress. The released suspension culture lignin binds several proteins which can be extracted with 1 M NaCl. Only peroxidases with pI > 9 are bound together with a second laccase-like oxidase. The N-terminal sequence of this oxidase has been obtained and cloning has been started. In addition, thaumatin/osmotin and glucan endo-1,3-b-glucosidase homologues are found bound to the lignin precipitate. To determine possible product specificities of the oxidative enzymes, dehydrogenation polymers (DHP) have been produced from coniferyl alcohol with both the oxidase and peroxidases. The substructures of formed polymer have been examined by NMR techniques. 1. Brunow G et al. 1990 Phytochemistry 29, 2535-2538 2. Brunow G et al. 1998 Phytochemistry 47, 1495-1500 3. Simola LK et al. 1992 Physiol. Plant. 84, 374-379

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Title : *PINUS PINASTER* - MOLECULAR BIOLOGY TOOLS FOR POLYMORPHISM DETECTION

Abstract : Maritime pine (*Pinus pinaster* Sol. ex Ait.) is a species of great economic interest to Portugal. An integrated pine-breeding program (Projecto PINUS) associating biotechnology with traditional breeding has been implemented to overcome problems related to wood deficit in the forest industry. The molecular characterisation of the 60 “plus” trees included in the Portuguese breeding programme was previously performed by the Pinus group. We verified that clones of the same trees, occasionaly, displayed distinct fingerprint patterns. In order to explain this observation we could admit that either those trees were mislabelled or that we are facing a situation of intraclonal variation. We performed microsatellite-primed PCR reaction (msp-PCR) using DNA samples of needles from three “plus” trees (2 clones each), collecting the needles at 3 branch levels. The results obtained point to labelling errors, although we have not yet excluded the possibility of superimposed intraclonal variation. Molecular markers technology was also used to identify the origin of different seed lots. For this work we have used two techniques, Cloroplastidial Single Sequence Repeats (cp-SSR) and Amplified Fragments Length Polymorphism (AFLP). AFLP, is a powerful technique, which allows a very good differentiation even when other methods fail. Seeds from 5 different Portuguese provenances were used to ascertain the value of above mentioned molecular techniques for certification. For each provenance, DNA pools from 10, 20 and 30 individual seeds were used for the PCR reaction. In each pool the DNA isolated from individual seeds was mixed in equal concentrations. Cloroplastidial microsatellites proved to be useful for this study of cytoplasmic diversity. These types of primers have been used in several forest species and have shown that the amplified fragments were highly polymorphic. Both cp-SSR and AFLP permitted the separation of seed provenances, samples from the same provenance being grouped together. However, the strategy has to be further improved to assess the optimal number of seeds to use in each reaction.

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Title : GENETIC ENGINEERING OF MARITIME PINE (PINUS PINASTER AIT.)

Abstract : Maritime pine covers more than 4 millions hectares in the Mediterranean region and, with about 1.4 million hectares, it represents the first coniferous species used for reforestation in France. Biotechnology programs implemented by AFOCEL in the past 10 years are expected to significantly speed up the long-term breeding programs initiated in the 1960's. In conjunction with cryopreservation techniques, development and integration of somatic embryogenesis in the improvement process could for instance greatly increase the availability of trees selected according to the requirements of wood chain industries. Moreover, such a promising tissue culture system undeniably provides a good target for genetic engineering and offers new prospects for rapid and efficient introduction of desirable traits, mostly unknown (e.g. herbicide or insect tolerance) or with low heritability in maritime pine (e.g. wood quality and processing, vigor). As a starting point, two different approaches were evaluated to transform embryonal suspensor masses initiated from selected seed families: the microprojectile bombardment technique (biolistic) and the Agrobacterium tumefaciens based method. In both cases, selection of transformed cell lines using the antibiotic hygromycine B was demonstrated to be highly efficient at no more than 10-20 mg/l within 4-17 (biolistic) or 4-19 weeks (Agrobacterium). We used a co-integration procedure of reporter uidA gene without intron (GUS activity) and selective hpt gene (hygromycine resistance) for biolistic experiments, whereas uidA gene with intron (suppressed GUS activity in bacteria) and hpt gene were present within the same T-DNA in Agrobacterium experiments. uidA and hpt were both under control of CaMV35S promoter. Stable integration and expression of reporter and selective genes was observed in 46-67% (biolistic) or 75-89% hygromycine resistant lines (Agrobacterium). In the latter case, Agrobacterium decontamination of most cocultured lines was achieved with 4 weeks maintenance on culture medium supplemented with 300 mg/l Augmentin, without any drastic effect on plant cell growth. Depending on experiment and Pinus pinaster genotype, transformation efficiencies were in the range 1.25-12.25 (biolistic) or 0-153 (Agrobacterium) transformed lines per gram tissue (fresh weight). Mature somatic embryos could be regenerated from several of these lines and transgenic plants expressing the GUS reporter gene in different tissues are currently growing in the greenhouse for further molecular and morphological evaluation. Key words: Pinus transformation, biolistic, Agrobacterium, transgenic tissues and plants

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Title : AGROBACTERIUM-MEDIATED DNA TRANSFER IN PINUS PINASTER AIT.

Abstract : As part of an integrated genetic engineering program of maritime pine (Pinus pinaster Ait.) developped by AFOCEL, an Agrobacterium-mediated DNA transfer procedure was evaluated to transform embryonal suspensor masses (ESM) originating from 6 genotypes (PN519, F311, E, A104, C115, S100) produced by controlled crosses of elite trees (unrelated families). We used the Agrobacterium tumefaciens strain C58pMP90 carrying the pCAMBIA 1301 plasmid vector (kindly supplied by L. Jouanin, INRA, France) to assess the efficiency of transformation. The multiple cloning site of this vector is located between reporter uidA gene (GUS activity) with intron (proximal to the right border of T-DNA transfer) and selective hpt gene (hygromycine resistance) so that transformed tissues and plants expressing GUS and showing resistance to the antibiotic hygromycine B will also have the cloned gene of interest. Following coculture of A. tumefaciens with ESM spread on filter paper, bacterial decontamination was achieved for 5 lines within 4 weeks maintenance on solid medium supplemented with 300 mg/l Augmentin. However, such a treatment was revealed to be ineffective for one Pinus line (C115), even after prolonged decontamination, suggesting genotype-related bacteria protection effect. Hygromycine resistant lines were obtained for 4 genotypes within 4-19 weeks of selection on culture medium supplemented with 20 mg/l hygromycine B. Maximum yields were regularly observed after 8-11 weeks on selective medium. Transformation efficiencies over independent experiments were in the range 18-156 (PN519), 0-23 (E), 0-1 (S100) or 0-0.1 (A104) transformed lines per gram ESM (fresh weight). No drastic effect of bacterial inoculum density and acetosyringone concentration during coculture could be detected on transformation efficiency. After 20 weeks selection, GUS expression was clearly revealed in about 82% hygromycine-resistant ESM lines and located in disseminated or tissue-organized embryo head (meristematic) and suspensor cells. In a selection of 22 early resistant lines (5-10 weeks on selective medium), 16 lines showed stable uidA expression over time (14-26 weeks), 3 only transient or irregular expression, and 3 no expression at all. Molecular PCR tests based on detection of uidA, hpt and Agrobacterium virulence gene (virD) concluded to the integration of both transgenes in 21 of these lines. Mature somatic embryos expressing the uidA gene were obtained from 7 putatively transformed lines and are currently grown for further analyses. Key words: Pinus transformation, Agrobacterium tumefaciens, transgenic tissues

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Title : GENOMIC ORGANISATION OF REPETITIVE SEQUENCES IN NORWAY SPRUCE GENOME

Abstract : In the frame of an EU project aimed at the analysis of the structure of the large Norway spruce (*Picea abies* Karst) genome (1C=15*10^9 bp), several different approaches were used. Assuming that such a huge genome is largely composed by repetitive sequences, 119 highly repetitive clones were isolated and sequenced. Their characterisation was performed using public sequences database homology searches, Southern blot hybridisation , copy number assessment and FISH experiments. Particular attention was paid to their methylation state; for this purpose two genomic libraries were constructed using different fractions of DNA digested with the restriction enzyme McrBC and several dot and Southern blot experiments were performed. Hybridisation onto garden blots, produced using genomic DNA from different *Pinaceae* species, as well as dot blot hybridisation, were carried out to study the conservation of *Picea abies* highly repetitive DNA families in the *Pinaceae* family. A protocol to extract high molecular weight (HMW) DNA was optimised. HMW DNA was then used to construct a BAC library. 3456 BAC clones, with an average length of inserts of 50 kb, were obtained and arranged in high density arrays onto nylon membranes. The filters were then hybridised with 16 probes representative of the various highly repetitive DNA families previously isolated and with two SSRs (polyAC and polyAG) probes. 1190 of the BAC clones showed strong signals when hybridised with our probes; in particular 390 of them showed strong signals when hybridised with 2 or more different probes. Twenty clones showing positive signals when hybridised with 3 or more different probes were digested with *EcoRI* and *HindIII* restriction enzymes and used to produce Southern blots in order to better investigate the relative arrangement of the different repetitive sequences at the microscale level. On the basis of previously described experiments few clones will be chosen and completely sequenced using a shotgun strategy.

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Title : MEXICAN WHITE PINES: MOLECULAR VARIATIONS AND TAXONOMIC DISTINCTIONS. FIRST STAGE.

Abstract : The mexican white pines include Pinus ayacahuite, P. strobiformis, P. strobus var. chiapensis, P. lambertiana, P. flexilis (var. reflexa?) in which molecular and morphological information is particularly scarce. A better understanding of the mexican white pines genetic resources and their genetic relationships is ssential for efficient conservation and subsequent utilization. Taxonomy and systematics are central to these efforts. Morphological information is not always adequate to assess relatedness and variation. Often, differences among species may be obscurate at the morphological level yet may be extensive at the molecular level. The objetive of this first stage is identify and select informative RAPD and ITS markers useful for understanding the taxonomic organization of this mexican white pines.

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Title : IDENTIFICATION AND APPLICATION OF TY1-COPIA RETROTRANSPOSON ELEMENTS IN OAKS (QUERCUS SPP)

Abstract : Various copies of the reverse transcriptase (RT) gene and the long terminal repeat (LTR) region of the Ty1-copia type retrotransposon sequences have been isolated from Quercus robur. Based on the isolated sequences a higher variation of the RT compared to the LTR region was observed within a single individual. Marked differences in hybridisation patterns after Southern analysisusing the RT region as probe were observed, allowing species differentiation (Q. robur, Q. pubescens, Q. dalechampii, Q. frainetto, Q. cerris) of oaks. Furthermore a sequence specific amplified polymorphism (S-SAP) system was developed using some isolated LTR sequences to study the genetic variation and relatedness of the closely related species/types of Q. petraea and Q. robur. Analysing individuals originating from a mixed population, no species specific retrotransposon insertion was observed, however the two species could be differentiated as sub-populations using statistical methods.

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Title : MULTILOCUS GENETIC STRUCTURE OF EUROPEAN CHESTNUT (CASTANEA SATIVA M.) HELLENIC CULTIVARS AND GENETIC DIVERSITY OF ORCHARD POPULATIONS

Abstract : Chestnut cultivation is very important in Greece for both nut and wood production, nevertheless insofar there has been no information on the numbers of cultivars used in grafted orchards as well as on the levels and structure of genetic variability in orchard populations in comparison to natural ones. In this study the clonal identification and multilocus genetic structure of chestnut cultivars from two Hellenic orchard populations originating from distant geographic locations is examined, while the levels of genetic diversity in the orchard populations are compared to the respective levels in natural chestnut populations of the corresponding location. Winter buds were sampled and the following enzyme systems were studied: alcohol dehydrogenase, diaphorase, isocitrate dehydrogenase 6-phosphogluconate dehydrogenase and shikimate dehydrogenase. A total of 6 loci and 11 alleles were revealed out of the 5 enzyme systems studied in a preliminary analysis. Results indicate that orchards are essentially multiclonal plantations in terms of their grafted part. The percent of unique genotypes was 15% in both orchards compared to 100% in natural populations. A total of 650 pairwise comparisons have led to the identification of four different cultivars (clones) that were present in each orchard population, while one clone unique to both populations. The comparison of genetic diversity indicators such as average number of alleles per locus, effective number of alleles percent polymorphic loci, observed heterozygosity, gene diversity and information indices suggest that there is a notable reduction in genetic diversity in orchard populations. On the other hand, the genetic variability within and between natural populations is significant and can be regarded as being among the highest reported for natural European populations. The implication of these results in breeding strategies is discussed.

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Title : GENETIC VARIATION IN *Nothofagus* SPECIES OF NORTHWESTERN PATAGONIA

Abstract : *Nothofagus nervosa* (Rauli) and *Nothofagus obliqua* (Roble pellin) are two native species of the rain forest of Argentina and Chile, being both part of the Andino–Patagónico forests. The Rauli and the Roble pellin have a very restrict distribution area in Argentina, specially the first, being the Rauli between 39° 20’ to 40° 40’ of latitud sur and the Roble pellin between 36° 50’ to 40° 15’. Both species are in simpatry between them and with *Nothofagus dombeyi* (Coihue). The Rauli and the Roble pellin were very exploitated in the past, specially the first, according to the good quality of their wood. These forests also suffered antropic alterations like fire following by livestock. These situations and the very restrict distribution of both species carrying our to study the genetic variation of them and their hybrids according to conserve their genetic source. In relation to our objective we studied 19 populations of Rauli along its distribution area in Argentina, using isoenzimatic markers, founding more genetic variation withing than between populations. We also found loci that are characteristic of certain populations. In Roble pellin we studied few populations until now, and with these results we could differentiat clearly these two species in relation with their pattern of bands in almost 5 enzymatic systems. Finally, we studied cpDNA in the populations of the north and the south distribution of Rauli, detecting one specific haplotype for each one of these two groups. These study carrying some implications in relation to the possible refugia of these species during the last glaciation. Rauli and Roble pellin hybridize naturally, we could find two specie specific markers with isoenzimes, and based on these we detected hybrid individuals in a progeny test. We registered different measures in these progeny test, specially height of the trees. The mean height of the hybrid trees were high than the mean of the two pure species, founding at the same time a very large variability in these parameter withing the two pure species and the hybrid individuals. The variability that show these species, specially Rauli, and their hybrids, suggest us to conserve a very important, or all, part of the distribution area that these species have in Argentina, and with these their genetic source.

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Title : SHIFTS IN CHLOROPLAST GENOME SIZE ACROSS PLANT LINEAGE.

Abstract : A study of the evolution of chloroplast (cp) genomes in higher plants has been initiated. It is based on the amplification of 25 relatively large (1-4 kb) cpDNA fragments with consensus primer pairs in 25 species (22 dicots, 2 monocots, and 1 gymnosperm). These 25 primer pairs are part of a set of 40 primer pairs that cover the entire Large Single Copy of the chloroplast genome of dicotyledons. First, the variation of the size of each amplified fragment was measured across species. As expected, a significant correlation between the coefficient of variation of the size of the amplified products and the percentage of intergenic spacer was found. Second, systematic trends of size variation of the amplified fragments across species were tested, by taking into account the phylogeny of the 25 species (it is based on the method of contrasts developed by Felsenstein for comparative data analysis). Significant directional trends of increase or decrease in size of the non-coding part of the genome were found. For instance Fagaceae tend to have larger cpDNA genomes, whereas Rosaceae tend to have smaller ones. The chloroplast genome could provide a unique model to study genome size evolution in organelles, because it is one of the best known genome and it is used extensively for phylogenetic reconstruction.

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Title : MULTILOCUS ESTIMATION OF GENE DIVERSITY IN TROPICAL TREES REVEALS IMPORTANT SPECIES DIFFERENCES

Abstract : Levels of genetic diversity were estimated for 8 forest trees of French Guiana which were selected because of their contrasting life history traits. Multilocus methods are used as RAPDs or AFLPs in order to assess genetic diversity at the whole genome level. However these markers suffer from a major drawback : they behave generally as dominant markers. Theoretical calculations were made to compare two different methods to assess genetic diversity with dominant markers, the phenotypic diversity (Hp) that considers presence and absence of fragments versus the genotypic diversity (Hg) that considers the alleles responsible for the presence or the absence of the bands. It is shown that these values (Hp and Hg) can only be compared in a limited number of cases, according to the frequency of the bands. The genetic monitoring of gene diversity is based on a two step sampling procedure: (i) the intra-locus sampling, e.g. sampling of trees and populations and (ii) the inter-locus sampling, e.g. sampling of loci within the genome. The associated components of sampling variance were estimated using resampling methods in order to make species comparisons. It is shown for both markers that the inter-locus variance exceeds by far (5 to 10 times) the intra-locus variance. Furthermore both components of the sampling variance are much higher for RAPDs than for AFLPs, even if both markers provide congruent rankings of the species. There are striking differences in the level of phenotypic and genotypic diversity among the species. Species that exhibit the highest level have large geographic distributions and are preferentially outcrossing, whereas species showing lower levels of diversity are endemic to the Guiana shield and exhibit strong genetic spatial patterns in Paracou.

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Title : GENE FLOW AND GENETIC DIVERSITY IN EUCALYPT PLANTATIONS AND NATIVE FOREST IN NORTH COAST NEW SOUTH WALES

Abstract : In Australia, plantation eucalypts have the potential to form intra- and interspecific hybrids with native trees from the surrounding local forest. One of the major plantation species grown for hardwood timber production is *Eucalyptus grandis* Hill ex Maiden (flooded gum). There is public concern that gene flow (seed dispersal and pollen movement) between these plantations and locally growing native trees of the same species may have impact on the genetic composition of the native forest. Gene flow via plantation pollen and seed may influence the genetic composition of native forest by the production of viable progeny that can compete and survive to reproductive maturity. This study will initially address the first two of these issues, whether there are significant differences in genetic diversity in material used in plantations in relation to the same species in adjacent native forests and attempt to detect and quantify gene flow. Broad-scale population structure of *E. grandis* has been assessed using a chloroplast marker. Other genetic markers such as microsatellites will be used to determine genetic diversity in plantations and native forest, and measure gene flow parameters in a planting of *E. grandis* in the North Coast region of New South Wales.

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Title : GENE FLOW IN A POPULATION OF A TROPICAL TREE *DICORYNIA GUIANENSIS* REVEALED BY PARENTAGE ANALYSIS USING MICROSATELLITES.

Abstract : Gene flow and other ecological processes such as competition, predation, herbivory and pathogen diseases, may significantly modify the initial spatial genetic structure at the population level. Theorical studies suggest that restricted gene flow reduces effective population size, and causes inbreeding depression (Slatkin 1985). Finally, estimating pollen-flow and seed-flow distances, and number of mating partners are important for the conservation of viable populations. Previous studies in tropical tree species were based on allozyme markers that appear to have low polymorphism. Since the development of molecular tools, highly polymorphic markers as microsatellites are available and useful for population genetics studies.
Gene flow and population genetic structure among individuals of Dicorynia guianensis, a tropical canopy tree species from French Guiana, were investigated in a 40-ha study plot. The estimation of gene flow was inferred from parentage analysis. Seven microsatellite loci were developed to identify the two parents of saplings. Our approach combines analysis of these biparentally inherited nuclear markers with a maternally inherited chloroplast marker for the mother identification. The polymorphism detected in previous studies of chloroplast diversity in this stand has been predicted to be sufficient for this analysis (Caron et al., 2000). In contrast, pollen donors of seeds collected this year from twenty adult trees were determined by paternity analysis using microsatellite markers.
Exclusion probabilities were estimated and parentage and paternity assignment were conducted using Famoz software program (Sophie Gerber – INRA Recherches Forestières – Laboratoire de génétique et amélioration des arbres forestiers). The genetic diversity was evaluated in each cohort and the multilocus outcrossing rate (tm) was estimated in each progeny. Investigation of spatial genetic structure showed significant correlation of genetic relatedness.
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Slatkin M (1985) Gene flow in natural populations. Annual Review of Ecology and Systematics, 16, 393-430.

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Title : COMPARISON OF GENETIC DIVERSITY ESTIMATES WITHIN AND AMONG POPULATIONS OF MARITIME PINE USING CPSSR AND AFLP DATA

Abstract : The nuclear and cytoplasmic genetic variation of 24 *Pinus pinaster* populations from two regions (France and Portugal) within the range of the species were estimated based on amplified fragment length polymorphism (AFLP) and chloroplast simple-sequence repeat (cpSSR) loci. The aims of the study were to assess the distribution of genetic diversity within and among populations with the two types of markers, to compare the genetic diversity estimates between provenances, and to compare the results derived from nuclear and cytoplasmic data. The genetic parameters estimated from AFLP and cpSSR loci indicated that there were high levels of genetic diversity in *P. pinaster*, which were mainly located within populations, and that the French and Portuguese provenances were clearly differentiated from each other. Populations' levels of diversity within provenances were found similar with the AFLPs, but not with the cpSSRs. The high inter-locus variance associated with the AFLP loci could account for the lack of differences in the former. Although AFLPs revealed much lower genetic diversity than cpSSRs, the levels of genetic differentiation (Gst) found with the two types of marker were similar, provided that loci showing less than four null-homozygotes, in any population, were pruned from the AFLP data. Furthermore, the Mantel test showed that the genetic distance matrix calculated using the AFLP data was highly correlated with the matrix derived from the cpSSRs. Despite the fact that the two types of marker have different modes of inheritance the trend found in genetic variation was the same, probably because gene flow through pollen exceeded the effects of genetic drift in shaping the genetic variation of the species at the geographical scale studied.

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Title : DIVERSITY STUDIES IN NATURAL POPULATIONS AND SEED ORCHARDS OF *P.SYLVESTRIS* USING MOLECULAR TOOLS

Abstract : In order to get an estimate on the impact of forest management on the diversity of Austrian Scots pine populations, different microsatellite markers (SSR) have been applied. 700 individuals out of 7 natural populations and one managed stand as well as 5 seed orchards have been investigated using nuclear and chloroplast SSRs. These two markertypes show a different mode of inheritance. cpDNA is inherited paternally and therefore is transmitted by pollen whereas the nuclear SSRs follow mendelian rules. Our results showed that the overall genetic variation and heterozygosity among populations is high, whereas genetic differentiation between populations is low. This might be due to the fact that in wind-pollinated species like coniferes, the outbreeding rate and geneflow is extremely high. Comparison of the genetic profile of 5 Austrian seed orchards to that of the investigated natural populations showed significant difference. The seed orchards aren't representing the genetic diversity found in natural populations. The selection of the plants for grafting according to their phenotype might have lead to the detected change in the genetic profile.

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Title : MONITORING *EUCALYPTUS GLOBULUS* AND *PISOLITHUS TINCTORIUS* GENE EXPRESSION DURING THE ECTOMYCORRHIZAL SYMBIOSIS DEVELOPMENT USING cDNA ARRAYS AND CLUSTER ANALYSIS

Abstract : Ectomycorrhiza is the result of a series of complex interactions leading to a finely tuned mutualistic symbiosis between a tree and a compatible soil fungus. Ectomycorrhiza formation and function alter both fungal and plant gene expression. The identification of a large number of novel genes expressed exclusively or predominantly in the symbiosis will contribute greatly to the understanding of the function of the ectomycorrhizal association. We have constructed a cDNA library of 4-day-old *Eucalyptus globulus*-*Pisolithus tinctorius* ectomycorrhiza and sequenced 950 clones obtained by random cloning and suppression subtractive hybridization. We have screened 715 arrayed cDNAs to identify symbiosis-regulated genes by using differential hybridization. Gene expression profiles obtained from free-living *Pisolithus tinctorius*, non-inoculated *Eucalyptus globulus* roots and ectomycorrhizas at various developmental stages, from early contacts to the functionning symbiotic organ, were analyzed. Comparisons of free-living partners and symbiotic tissues revealed significant changes in the expression levels (differential expression ratio > 2.0) for 11 to 23% of the genes analyzed at the different stages of mycorrhiza formation. No ectomycorrhiza-specific gene was detected. We have derived groups of coordinately expressed genes (i.e. regulons) using clustering algorithms (self-organizing maps, *k*-means, hierarchical clustering). The main fungal regulons contained genes coding for cell-wall and membrane proteins, communication genes, and metallothionein-related protein encoding genes. In the host root, a major down-regulated regulon comprised genes involved in water transport and stress (MIP and TIP aquaporins ; ABA and water-stress induced proteins) suggesting that mycorrhiza development improves water uptake. Clustering analyses allowed us to distinguish three main expression profiles during mycorrhizal development: early up-regulated genes (e.g. *Pisolithus* hydrophobins), genes preferentially up-regulated in the mature symbiotic organ (e.g. *Eucalyptus* extensins) and genes up-regulated over the whole developmental process.

Key words: cDNA-arrays - *Eucalyptus* - Ectomycorrhiza - Expressed Sequence Tags - Regulon - Symbiosis-Regulated Genes - Water-stress proteins.

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Title : GENOMICS OF WATER USE EFFICIENCY IN MARITIME PINE

Abstract : Drought adaptation features have practically not been considered in tree breeding programs so far, mainly due to the complex nature of drought resistance. In response to this lack an interdisciplinary effort was recently developed to investigate the physiological, genetic and molecular components of maritime pine (Pinus pinaster Ait.) response faced to drought. A part of this effort was the production and analysis of about 2000 ESTs. Briefly, roots and needles were sampled from seven week old seedlings raised in hydroponic solution. Water deficit stress was obtained by adjonction of Polyethylene Glycol, as an osmoticum, in the growing medium (-0.45 Mpa). Four cDNA libraries were constructed from these tissues from which 2688 clones were partially sequenced. This project provided us with a catalog of about 1300 Pinus pinaster genes and an estimate of the abundance of each gene in each tissues and drought status. The variation in the relative frequency of each ESTs was then used to point out the differential expression of the corresponding gene following the concept of « digital Northern » comparision (Audic and Claverie, Genome Research 7:986-995). The software and the Web interface to access it are to be found at http://igs-server.cnrs-mrs.fr/~audic/significance.html. The results of this project are gathered into a database with those those that will be provided by the Lignome Project. The Lignome Project, funded by INRA aims at producing ESTs from woody plants of interest : poplar, pine, oak, apricot, peach and vine. We will focus, during the Lignome Project, on water use efficiency in maritime pine by analysing more than 20 000 roots ESTs from the same libraries.

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Title : WOOD PROTEOMICS IN MARITIME PINE

Abstract : Differences in wood characteristics within a single tree are a common feature. These include: (i) variation within annual ring in temperate zones, i.e. early vs. late wood, (ii) variation due to juvenile wood with extremely variable properties ranging from the core to the bark particularly in the early years of cambium activity, and (iii) variation between normal and reaction wood. These 6 types of wood possess distinct chemical, anatomical and physical characteristics. This variability makes it possible to correlate gene/protein expression profiles with wood properties, and ultimately to identify candidate genes involved in the genetic control of wood quality and end-product properties. Recent improvements in realisation of two-dimensional gel electrophoresis (e.g. immobilized pH gradient) and protein characterisation (mass spectrometry) allow considerable progress in proteome analysis. Although not very used in plant genomics, we will show that these advances afford opportunities to have an overlook at gene product expression and address physiological question, at a level that is much relevant than mRNA.

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Title : ANALYSIS OF WOOD DEVELOPMENT IN BIRCH BY cDNA SEQUENCING

Abstract : In forest trees, wood formation and stem diameter growth result from the activity of the vascular cambium. Development of xylem and phloem involves several fundamental processes of plant growth and development including cell division, cell expansion, formation of secondary cell walls and programmed cell death. During these developmental steps (involving cellulose, hemicellulose, and lignin biosynthesis), most of the structural and chemical properties of wood and fibre are determined. To study gene expression in the wood developing tissues we have constructed EST libraries representing three distinct zones of the cambial region of an actively growing ten-years-old tree. The EST libraries were generated by inserting the cDNAs into Uni-ZAP XR vectors and then excising them to pBluescript phagemids. By using PCR and automatic sequencing methods in 96 well format, large amounts of clones have been sequenced from the 5 prime end of the clone and the sequences collected into birch EST database. The average insert size in the libraries is about 600 base pairs. At the moment about 12500 sequences have been collected to a local birch database: 5012 sequences representing the phloem side of the cambium; 4645 sequences representing the xylem side and 2760 sequences representing a later stage of xylem development. A total of 7577 unique transcripts (4459 appearing once and 3118 twice or more) were identified. About 66% of the EST sequences show similarity to previously described sequences in public databases. Among the sequences most of the currently known steps of lignin and cellulose biosynthesis are represented. Interestingly, several genes specifically abundant in a distinct cambial zone are discovered. In combination with existing genetic stocks of various birch trees, the birch EST libraries and database will be valuable resources for forest research directed understanding the genetic control of wood formation.

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Title : MICROARRAY ANALYSIS OF EXPRESSION DIFFERENCES BETWEEN WILD-TYPE AND CAD NULL MUTANT LOBLOLLY PINE

Abstract : Mutants provide special advantages for the analysis of gene expression on microarrays. For example, a mutant in a gene coding for a biosynthetic pathway enzyme could affect the transcription of other pathway genes via feedback regulatory mechanisms. The detection of co-regulated suites of genes is one of the most important goals of expression analysis. In loblolly pine, the expression of the gene encoding cinnamyl alcohol dehydrogenase (cad) is almost completely knocked out for one of the two alleles in breeding program selection 7-56. In this study, we analyzed gene expression in selfed progeny from 7-56 using an array with 384 pine ESTs. Samples of RNA were taken from differentiating xylem, needles, and shoot tips from two wild-type homozygote trees and two cad null homozygote trees. The preliminary analyses showed that the differences in expression for lignin and non lignin pathway genes were subtle. Further analysis with more powerful statistical methods and additional replication are underway to provide greater resolution to detect differences.

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Title : GENE EXPRESSION PROFILING IN DIFFERENTIATING XYLEM OF LOBLOLLY PINE

Abstract : The formation of secondary xylem is a biological process of both ecological and economic importance. Over 40,000 xylem ESTs have now been sequenced from loblolly pine, the most important commercial forest tree species in the U.S. (web.ahc.umn.edu/biodata/nsfpine). Our goal is to learn more about regulatory, biosynthetic and cellular processes involved in secondary xylem differentiation by characterizing gene expression profiles. These studies aim at a broad understanding of the roles of newly discovered pine genes in secondary xylem formation and identifying candidate genes that potentially control specific wood properties. We are currently conducting preliminary experiments with ~350 abundantly expressed pine xylem ESTs; comparing glass slide microarray and membrane array systems to optimize methods. The natural variability of wood properties affords opportunities to correlate transcript profiles with wood properties. For example, we are comparing transcript profiles of differentiating xylem at different times during the growing season, corresponding to early wood and late wood. Wood specific gravity is known to vary significantly between these two phases of xylem formation in loblolly pine. Cell wall thickness ranged from 3 mm (in early wood) to 8 mm (in the late wood) within a single growth ring and differential expression of specific ESTs may correlate with cell wall thickness. Other opportunities to investigate the relationship between wood properties and gene expression include comparisons of juvenile and mature wood, compression and normal wood, as well as the analysis and genetic variability among populations and in pedigree families.

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Title : INEXPENSIVE, HIGH DENSITY H.A.P.P.Y. MAPS OF TREE GENOMES – A PROPOSAL

Abstract : HAPPY mapping is a simple, rapid, cost-effective, cloning-free, PCR-based procedure for making high-resolution genome maps. This method has been applied for high-resolution, error-free mapping in a wide variety of genomes, including human, *Tetraodon*, *Dictyostelium*, and a menagerie of parasite genomes. HAPPY mapping is being used to assemble the shotgun sequencing data of *Cryptosporidium parvum* and *Dictyostelium discoideum* genomes. The procedure has now been validated for mapping plant chromosomes and genomes by reconstructing the physical map of a 1.8 Mbp region around the FCA locus of *Arabidopsis thaliana*. The resulting map, spanning around 10% of chromosome 4, is in excellent agreement both with the STS content of mapped BAC clones spanning this region and with the DNA sequence, and has a mean marker spacing of 16 kbp. We conclude that HAPPY mapping is a uniquely valuable addition to currently available plant genome mapping tools. Based on our results and on the current understanding of plant genome architecture, we argue that HAPPY maps can be made immediately and with little effort for most plant species and, further, that such maps can greatly aid the construction of genome-wide or regional physical maps. HAPPY mapping provides an unique opportunity to revisit issues in genome mapping and to reassess the hitherto impossible challenges of cost-effective mapping of plant genomes and genomes of plant pathogens and beneficial organisms like mycorrhizal fungi. A thumb-nail scenario and proposal for cost-effective mapping of European Union-funded tree genomes and the genome portfolio of the International Union of Forest Research Organizations is presented.

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Title : LIGNOME: THE FRENCH FOREST TREE GENOMICS INITIATIVE

Abstract : In 2001 INRA launched the LIGNOME projects to introduce the new tools of genomic science in perennial plants : forest trees, fruit trees and grape. Here we describe the forestry component of this project. It focuses on three species (pine, poplar and oak) selected for their great importance in the French forestry-wood chain. Application of genomic sciences in these long-lived organisms will improve our understanding of their unique biology and will accelerate the discovery of genes controlling economically and ecologically important traits. Application of these technologies are expected to increase selection efficiency and/or reduce the time and costs associated with measuring such traits, as well as to improve the management of forest tree resources.

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Title : GENETIC ANALYSIS OF DISEASE RESISTANCE IN *POPULUS*

Abstract : Poplar is one of the most planted tree species in Europe. Due to the long generation times of trees, the genetic improvement of trees by conventional breeding lags far behind that of annual plants. The possibility to generate genetic maps allows to unravel the genetics of particular traits and to identify the genes behind these traits. This new information can be of great value to improve classical breeding programs and opens possibilities to further improve elite genotypes by genetic engineering. Three genetic maps of poplar (P. deltoides, P. trichocarpa and P. nigra) have been constructed using the two-way pseudo-testcross strategy in combination with AFLP. The maps were generated from 2 controlled crosses sharing the same female parent (P. deltoides cv. 'S9-2' x P. nigra cv. 'Ghoy' and P. deltoides cv. 'S9-2' x P. trichocarpa cv. 'V24'). Microsatellite markers (SSR), made available through the Poplar Molecular Genetics Co-operative (PMGC), were used to align the three maps. These maps are now used to study the genetics of disease resistance, the major selection criterion for poplar breeding. Melampsora larici-populina is one of the most damaging fungal pathogens for poplar in Europe, and both quantitative and qualitative resistance have been recognised. Molecular markers, associated with qualitative resistance to M. larici-populina have been identified and a fine-map has been constructed around the resistance gene as a start point for positional cloning. For this, a binary BAC library has been constructed from a hybrid containing the resistance gene. To reveal loci possibly involved in polygenic resistance, BACs containing disease resistance (NBS-LRR-class) gene-like sequences were identified by BAC library hybridisation, and are currently being mapped. In parallel, QTL analyses for polygenic resistance are carried out. The sequence of one BAC, flanking the M. larici-populina resistance gene, has been determined and annotated, and reveals that the MER genes are present in a large cluster of NBS-LRR type disease resistance genes.

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Title: IMPROVING FRAXINUS (ASH) PRODUCTIVITY FOR EUROPEAN NEEDS BY TESTING, SELECTION, PROPAGATION AND PROMOTION OF IMPROVED GENETIC RESOURCES

Abstract: Genetic resources of Fraxinus will be characterised by recording existing provenance, progeny and population tests. A new provenance trial of material from the European natural range will be established in six countries. Genetic diversity estimates will be by already developed polymorphic microsatellites and cp DNA markers. Gene flow estimates will be evaluated via paternity analysis in pure and hybrid stands. The best populations and provenances will be identified to aid in Certification. Selected individuals and tested progeny will be propagated vegetatively. Physiological, biochemical and physical treatments will be optimised. These positively affect micropropagation, flower induction and propagation by cuttings to give high quality plants on a large scale for clone/variety testing by SMEs and deployment to farmers/foresters. Alternative models and means of diffusing improved material from the development phase to the end users will be critically examined in conjunction with user groups.

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