

# Dendrome

## Forest Tree Genome Research Updates

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### Biotechnology Research at the North Central Forest Experiment Station

Charles H. Michler  
Project Leader, Genetics and Pathology

North Central Forest Experiment Station,  
Forestry Sciences Laboratory, 5985 Highway K,  
Rhineland, WI and 1992 Folwell Ave., St. Paul, MN 55108.  
Email : michlerc@newnorth.net

Biotechnology research is performed in two research work units within the Station: Rhineland, WI (Forest Genetics) and St. Paul, MN (Forest Pathology). The mission of the program is four-fold: 1) to develop molecular genetic tools to assist in our understanding of the genetic structure of tree and pathogen populations; 2) to identify genes for important quantitative traits; 3) to develop micropropagation technologies for production of elite trees; and 4) to develop gene transfer technologies for introduction of novel genetic constructs for disease resistance studies. Objectives within the program mission focus on genetics of eastern white pine (*Pinus strobus*), white pine blister rust (*Cronartium ribicola*), northern red oak (*Quercus rubra*), butternut (*Juglans cinerea*), butternut canker (*Sirococcus clavignenti-juglandacearum*), aspen (*Populus spp.*) and septoria canker (*Septoria spp.*).

As part of our pine genetics program, Craig Echt (cecht@newnorth.net) and Paula Marquardt (pmarquar@newnorth.net) have been developing simple sequence repeat (SSR, microsatellite) markers. A number of (AC)<sub>n</sub> repeat primer pairs are now available from Research Genetics Inc. and additional markers for other repeat motifs are expected to become available this coming year. The goal is to provide an informative set of publicly available SSR markers for use in white pine genetics. Loblolly pine SSR markers are being developed by John Erpelding (jerpeldi@newnorth.net), a newly hired post-doc. Once developed and evaluated, the loblolly pine primer pairs will be available to university and government researchers upon request to International Paper Co., who is sponsoring the research.



*Cupressus sempervirens var horizontalis* (Mill.) in Koprulu Canyon National Park, Antalya, Turkey  
photographer: Zeki Kaya

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The eastern white pine markers have been tested for use among hard pines, but appear to be informative only in other white pine species, e.g. *P. lambertiana*. Specific information about individual SSR markers will soon be made available on the Dendrome server. In collaboration with Dr. Giovanni Vendramin (CNR, Firenze, Italy) and Dan Pubanz (Menominee Tribal Enterprises, Wisconsin), nuclear and chloroplast SSR markers are being used to study population genetic diversity and structures among natural and managed eastern white pine stands on the Menominee Indian Reservation.

Long term research goals include plantation establishment of selfed eastern white pine progeny for use in mapping some components of fitness and for studying the genetics of white pine blister rust resistance. One plantation currently contains three selfed families, represented by a total of 400, four-year-old progeny. Next year, an additional 1000 progeny from both self and reciprocal crosses obtained from various clones used in the blister rust program will be planted.

The white pine blister rust program, under the direction of Paul Zambino (paulz@puccini.crl.umn.edu), has three objectives: 1) to determine rust variation in the Lake States USA and compare it with rust variation in the rest of N.

America; 2) to determine if resistance can be reliably identified in eastern white pine seedlings inoculated at an early age; and 3) to determine whether there is a race-specific component to seedling resistance. PCR-based molecular DNA marker methods (RAPD fingerprints, RAPD-SSCP) will be used to determine genetic diversity. Codominant markers (RAPD-SSCP) will enable detection of heterozygosity within single genotype strains previously purified by increase from single spores. Pure strains from genetically distinct populations will be used to inoculate seedlings. Although pine seedlings currently being inoculated are from open pollinated families derived from clonal parent trees with known or suspected resistance or susceptibility, a diallel series of crosses and selfs among selected parents have been made for future studies. Sugar pine with and without the MGR gene have also been included in each inoculation to detect one known form of race-specific virulence. Once resistant white pine families are identified, resistant individuals will be cloned and tested in greenhouse and field trials to determine if resistance will be general against a diverse sample of strains, and to capture additional races to be used in future screening.

In support of our clonal propagation and genetic engineering program, Charles Michler (michlerc@newnorth.net) and Paula Pijut (pijut001@maroon.tc.umn.edu) have developed improved micropropagation technologies for eastern white pine. These protocols are being used for both mass propagation of elite genotypes and within Michler's genetic engineering program. In a collaborative effort with John Davis (jmd@nervm.nerdc.ufl.edu) eastern white pine chitinase genes have been isolated and work is currently focused on isolating the promoter regions from one chitinase gene. The goal is to determine promoter function including tissue specificity and thereafter develop efficient genetic engineering protocols for pines using constructs with pine promoters. Eastern white pine seedlings are currently being tested in greenhouse studies to determine stability of transgene insertion.

Pat Tomlinson (tomlinsp@newnorth.net) has initiated a research program that will use PCR-based molecular markers (inter-SSR) to distinguish trees that are either northern red oak (*Quercus rubra*), northern pin oak (*Q. ellipsoidalis*), or hybrids between them. Northern red oak is preferable for timber, but the species hybridize easily. Species identity is important to nursery and land managers for reforestation efforts. In addition, this work will be important for predicting future shifts in oak populations that could result from climate change since northern pin oak is more drought tolerant. A study site has been identified which visually shows a gradient in edaphic characteristics consistent with the presence of a gradient between these two species.

## Dendrome

Dendrome is a bi-annual research update on forest tree genomes. If you would like to submit an article, please do so by March 15, 1997. Please include your name, address, fax number or email address. Also any type of graphic or tree picture is welcomed.

Editor

David Neale

[dbn@s27w007.pswfs.gov](mailto:dbn@s27w007.pswfs.gov)

Design/Production

Kim Marshall

[kam@s27w007.pswfs.gov](mailto:kam@s27w007.pswfs.gov)

For information contact:

[dendrome@s27w007.pswfs.gov](mailto:dendrome@s27w007.pswfs.gov)

or write:

Dendrome,

Institute of Forest Genetics,

P.O. Box 245,

Berkeley, CA 94701, USA

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Mike Ostry (ostry001@maroon.tc.umn.edu) is using molecular fingerprinting of aspen to determine resistant genotypes within a pedigreed aspen population for his hypoxylon canker early screening program. Hypoxylon canker is a serious disease of native and planted aspen and this effort is in support of the University of Minnesota Aspen Tree Improvement Cooperative. In work with other *Populus* species and important pathogens, Ostry and Kathy Ward, have developed RAPD markers to distinguish between *Septoria musiva* and *S. populicola* and to determine genetic variation within species. *S. musiva* has not been found in the Pacific Northwest, but from Ostry's studies, it has been determined to be significantly more damaging than the native pathogen, *S. populicola*, which causes only minor leaf damage. Thus, it will be important to prevent overland transmission of this pathogen.

In addition to her eastern white pine micropropagation research, Pijut has developed protocols for somatic embryogenesis, clonal micro-propagation, and shoot regeneration from callus for the forest tree species, butternut (*Juglans cinerea*). Butternut is a fine hardwood species whose future existence is threatened by butternut canker disease (*Sirococcus clavigignenti-juglan-dacearum*) and poor natural regeneration. These vegetative propagation tools will be used to mass propagate and to aid *in vitro* screening of putative canker resistant trees. In addition, Ostry is developing molecular markers to distinguish between pathogen strains and is developing *ex vitro* screening procedures to test host resistance and host range across other *Juglans* species.



### Forest Tree Genome Research at the Canadian Forest Service - Atlantic

Linda DeVerno, Judy Loo, Donny McPhee, Alex Mosseler, Kurt Johnsen, John Major, Tannis Beardmore, Yill Sung Park, Dave Barrett, Jan Bonga, Ron Smith, Stewart Cameron

CFS-Atlantic, Fredericton, New Brunswick, Canada E3B 5P7  
Email : ldeverno@fcmr.forestry.ca



The dust is beginning to settle after the major cutbacks and layoffs in the Canadian Forest Service following the 1995 federal budget. Closure of the Petawawa National Forestry Institute resulted in researchers being transferred across the country to each of the five remaining institutes. Science networks have been established to address national and international forestry issues in a manner that links science with government policy. Each of the five forestry institutes in Canada has become the lead centre for one or two networks, although research in several networks is being conducted at each institute, with the goal of developing an integrated nationwide approach. Canadian Forest Service - Atlantic, based in Fredericton, New Brunswick, is the lead centre for the Biodiversity and Forest Health Networks, with substantial research also being

conducted in the Tree Biotechnology and Advanced Genetics Network, as well as several other networks.

#### *Forest Genetics Research - Biodiversity Network*

The objectives of the Biodiversity Network are to monitor and report on the status of forest biodiversity, assess the impacts of forest management and other pressures on biodiversity, and to study and promote means to conserve biological diversity of forests. Several studies are being undertaken to assess genetic diversity in a variety of forest ecosystems. Researchers involved in genetic studies in the Biodiversity Network include T. Beardmore, L. DeVerno, K. Johnsen, J. Loo, J. Major, D. McPhee, and A. Mosseler.

Several tree species have experienced recent population declines due to

harvesting practices and/or disease in certain parts of their geographic range. The consequences of small population size, species density, and population fragmentation are being assessed using isozymes to measure genetic diversity and inbreeding in small, isolated populations of white pine, pitch pine, white spruce, and bur oak. Results of these studies will provide estimates of minimum viable population sizes and population genetic structure that can be used to develop forest management guidelines to conserve genetic diversity and reproductive fitness.

Molecular markers such as RAPDs and RFLPs can be used to monitor and assess the status of forest biodiversity. Genomic analysis of selected tree species will identify individuals and populations that

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possess unique genetic material that should be considered for conservation. The RAPD technique has been used to identify polymorphism in the red pine genome as well as several other tree species. The potential for using RAPD markers to identify hybrids of various species is also being investigated.

The *ex situ* conservation of rare and endangered tree species depends on the ability of seed to tolerate storage. Twelve of the 16 tree species listed as rare and endangered in Canada cannot be stored for longer than 2 years and viability declines rapidly during storage. Characterization of the genetic components of seed development and the biochemical processes associated with the ability of the seed to tolerate desiccation are being studied by protein electrophoresis and RT-PCR. Strategies and treatments are being developed to ensure a reliable supply of high quality seeds for conservation and forest regeneration.

Two new collaborative studies will represent major research endeavors for the biodiversity project. Red spruce, whose populations are on the decline in Atlantic Canada, is being studied to assess the need for active gene conservation management. Population, physiological, and ecological genetic and demographic studies are being initiated. Ultimately, a gene conservation strategy will be developed, if needed. The objective of the second collaborative project is to facilitate conservation of endangered, rare or threatened species and populations of the piñon pines in Mexico. The project involves researchers from CFS-Atlantic, Mexico, and the U.S. and will assess the genetic and demographic status of species and populations. Natural and human-induced threats

will be determined and appropriate gene conservation strategies will be developed.

#### *Tree Genome Research - Tree Biotechnology and Advanced Genetics Network*

The four main objectives of the Tree Biotechnology and Advanced Genetics Network are i) to characterize important commercial and adaptive traits to develop advanced genetic tools for more efficient breeding programs, ii) to develop new genetic engineering methods for tree improvement and protection, iii) to integrate somatic embryogenesis into operational reforestation programs, and iv) to develop deployment strategies for genetically improved trees. Researchers involved in this Network include T. Beardmore, D. Barrett, J. Bonga, S. Cameron, L. DeVerno, Y-S. Park, S. Pond, and R. Smith.

Alterations of tree genomes in the cell culture system are important to the understanding of processes such as induction of embryogenesis, maturation, and regeneration. The process of culture and regeneration should result in the production of clones that are phenotypically and genetically identical to the original material, which is important for large-scale clonal propagation of improved tree genotypes. Genetic stability and somaclonal variation of somatic embryogenic cell lines, cryopreserved tissue, and regenerated plants are being assessed using RAPD markers. Molecular markers are also being developed to differentiate between embryogenic and non-embryogenic

cell lines using differential display RT-PCR, to aid in early selection of genotypes with high embryogenic potential. Involvement of peroxidases in somatic embryogenesis of jack pine and white spruce is being evaluated using the patterns to determine if expression of these genes could be an early molecular marker for embryogenesis. In addition, controlled crosses are being evaluated to determine if molecular markers can be used to tag specific genotypes with high embryogenic potential.

CFS-Atlantic is also cooperating with CFS-Quebec in a project under the National Biotechnology Strategy Fund entitled 'Molecular Biology of Conifers for Flower Sterility'. The main objectives of this project are to understand conifer reproduction at the molecular level and to produce sterile transgenic trees using recombinant DNA technology. Research towards the isolation and characterization of homeotic genes in black spruce is ongoing at CFS-Quebec, while work to-date at CFS-Atlantic has focused primarily on using *in situ* hybridization to identify gene expression associated with the differentiation of primordia (signals for buds to develop reproductively) and for identifying sex-specific gene expression. This molecular biology work complements an ongoing research program on the cell biology and physiology of conifer reproduction. Once putative sterile lines are produced, CFS-Atlantic will be active in applying accelerated growth and flower induction techniques to confirm whether they are in fact sterile.



# Molecular Biology of Drought Tolerance and Transformation of *Populus* and *Pinus* at the Hebrew University of Jerusalem



Arie Altman, Tal Alegrand, Dan Pelah, Tzvi Tzfira, Basia Vinocur, Wangxia Wang and Ophir Yarnitzky

The Otto Warburg Center for Agricultural Biotechnology, Faculty of Agriculture of the Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. Fax: X-972-8-9468263; E-mail : Altman@agri.huji.ac.il

The laboratory of plant biotechnology of Arie Altman is engaged in several aspects of forest tree biotechnology research. This includes: (1) Molecular analysis of drought tolerance and tree responses to water stress, (2) *In vitro* clonal propagation, and (3) Tree transformation. The studies involve several *Populus* species and clones (especially aspen, *P. tremula*) and *Pinus* (especially the Aleppo pine, *P. halepensis*). The research program is supported by university, national and international funds, and parts of it are in collaboration with Oded Shoseyov, Alexander Vainstein and Meira Ziv - all from the Faculty of Agriculture, and with Dorothea Bartels of the Max-Planck-Institute, Köln.

## *Molecular analysis of water stress and drought tolerance:*

In an earlier study, we have identified a novel protein (BspA) that is highly expressed in cultured shoots of aspen (*Populus tremula*) in response to gradual water-stress. This 66 kDa protein, identified as boiling stable, was highly expressed as early as 1 hr after drought treatment. The protein accumulated during progressive water stress, decreased upon rehydration, and was expressed in response to ABA application, as detected by SDS-PAGE protein and western blot analyses. Anti-BspA antibodies also cross-reacted with another protein, having a molecular mass of 119 kDa. Although the 119 kDa protein was also induced by water stress, it was detected only in the total protein, not in the boiling-stable fraction. BspA cross-reacted with antibodies raised against another water-stress-responsive protein isolated from the African resurrection plant *Craterostigma plantagineum*. The N-terminal amino acid sequence of BspA was determined, and exhibited high homology to wheat germins GF-2.8 and GF-3.8. BspA was the only major water-stress-responsive boiling-stable protein detected in aspen.

Other water-stress-responsive proteins - DSP 16 (dehydrin), cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and sucrose synthase - were then studied in aspen roots and shoots. These proteins were identified immunologically using polyclonal antibodies isolated from the resurrection plant *Craterostigma*

*plantagineum*. A 43-kD homologue of dehydrin was constitutively expressed in water-stressed roots and shoots of aspen, while a 31-kD homologue accumulated only in the shoots. Shoots and roots of intact plantlets subjected to ABA application accumulated a 33-kD dehydrin homologue. A sucrose synthase homologue (90 kD) accumulated in water-stressed shoots and was constitutively present at high levels in control and water-stressed roots harvested from intact plantlets. GAPDH, a key enzyme in the glycolysis and gluconeogenesis pathways, exhibited some accumulation in shoots in response to water stress. Sugar analysis revealed that water stress results in increased sucrose and decreased glucose levels in the aspen leaves. These data suggest that the water-stress-response mechanisms present in herbaceous plants are also present in woody plants.

In an attempt to characterize *Populus* genotypes which differ in their water-stress response, we studied the accumulation of BspA, and of the water-stress related protein dehydrin dsp-16 and sucrose synthase from the resurrection plant *Craterostigma plantagineum*, in two greenhouse-grown *Populus* clones. Detached leaves of *Populus tomentosa* lost more water than *Populus popularis* resulting in a significant decrease in leaf water potential. Using electrolyte leakage analysis, it was found that detached leaves of *Populus popularis* are more tolerant to water-stress than detached leaves of *Populus tomentosa*. Using western blots with the corresponding antibodies, we have found in *Populus popularis* accumulation of BspA and sucrose synthase due to water stress, and constitutive presence of a dehydrin-like protein. In contrast, a low expression of BspA was found in *Populus tomentosa*, but not of sucrose synthase and dehydrin-like proteins. Desiccation tolerance in many tissues can be partly attributed to soluble sugars. Analysis of the amount of soluble sugars did not reveal clear cut differences between the two clones, except for significant sucrose accumulation and glucose reduction in water stressed *Populus tomentosa* and an increase in glucose in water stressed *Populus popularis*. This data suggests a positive correlation between increased

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water stress tolerance of one poplar clone as compared with another and accumulation of water stress related proteins and sucrose synthase.

Recently, further characterization of BspA and cloning of the BspA gene of aspen was achieved. Ion-leakage tests and western blots were performed to further characterize respectively, the possible function and structure of this protein. A positive correlation was found between BspA accumulation and reduced ion leakage from leaves following water stress. Due to N-terminal blocking, internal fragments of this protein were analyzed after tryptic digestion, and the gene encoding BspA was isolated and sequenced. The BspA gene was found to encode a 12.5-kD hydrophilic polypeptide. Thus the original BspA polypeptide taken for antibody preparation and internal cleavage had a higher molecular mass (66 kD) than the protein encoded by BspA. Northern blot analysis revealed that BspA is encoded from a small mRNA (0.8 kb) which is constitutively expressed during cold and water stresses. We suggest that BspA is regulated at the post-transcriptional level, for two reasons: the small 12.5-kD protein was not detected by anti-BspA antibodies, before or after stress, and BspA stained positively with PAS, indicating it to be a glycoprotein. As for its cellular localization, BspA was found in both soluble and non-soluble cell fractions. *In situ* immunogold staining localized BspA within parenchyma and pith cells of stems, and in the vascular bundles of leaves. To the best of our knowledge, this is the first report describing the isolation of a post-transcriptionally regulated heat-stable water-stress-responsive protein, the level of which is positively correlated to reduced membrane leakage.

#### *Clonal in vitro propagation:*

For continuous micropropagation of aspen and other *Populus* clones, shoot explants are routinely subcultured every 5-6 weeks on half-strength MS supplemented with 500 mg/l casein enzymatic hydrolysate, at 25°C under a 16-hr photoperiod using cool-white fluorescent light (50-60 mmol/m<sup>2</sup>/sec). For bud regeneration in *Pinus halepensis*, excised mature embryos were cultured in an inverted position on 0.5 MS medium supplemented with cytokinins and auxins, then placed in their normal position for further growth, and rooted. Alternatively, the resulting adventitious buds served as a source for needles. Elongated needles were excised and placed horizontally on 0.5 MS medium with different concentrations of BA and/or ABA. In addition, 3-year-old pine trees were pruned and sprayed with BA or TDZ to induce fascicular buds, and the resulting elongated shoots were then used for *in vitro* culture and bud regeneration. For somatic embryogenesis, mature pine embryos were

grown on a modified DCR medium. Procedures for massive induction of adventitious shoots from mature pine embryos were thus refined, and a high rate of first and second “generation” adventitious buds was achieved. These buds were rooted and plantlets are being established.

Using an alternative approach, the effect of pruning and cytokinin application on rejuvenation and multiple bud formation was studied in 3 year old pine trees. After pruning, the trees were sprayed with either TDZ or BA, and a very high number of buds were induced by the applied cytokinins. Most of the developing buds originated from brachyblasts, BA inducing well-formed and elongated buds, either single or multiple (clusters consisting of 2-8 buds). TDZ, on the other hand, induced the formation of mostly multiple buds, in clusters containing 10-80 buds, of which only one bud elongated. The resulting shoots have been rooted and produced a considerable number of pine plantlets.

#### *Transformation procedures and transgenic trees:*

Separately, we have established and refined a transformation system of poplar and pine, using an *Agrobacterium rhizogenes* and *A. tumefaciens*-based vector. Regeneration from stem, leaf and root explants has been established, and expression of GUS, transient and stable, in transgenic plants was found, as detailed in the following.

The natural ability of aspen (*Populus tremula*) roots for direct shoot-bud regeneration was harnessed to establish a highly efficient transformation and regeneration procedure that does not require a pre-selection stage on antibiotics. Aspen stem segments were transformed using a wild-type *A. rhizogenes* (LBA9402), with the binary p35SGUSINT plasmid. This plasmid carries the genes coding for GUS and NPT II. High levels of transient GUS expression were found in the basal cut surface of 87 % of the segments, and 98 % of these formed well-developed adventitious roots. Proliferating root cultures were established in liquid culture, and GUS expression was found in 75 % of the roots. Shoot-bud regeneration in root cultures was very high: 99 % of the roots yielded buds, of which 91 % expressed GUS. Southern blot analysis and PCR confirmed the transgenic nature of the plants expressing GUS. Kanamycin resistance of transformants was tested with respect to callus growth and bud regeneration. Callus from transgenic plants showed a high growth rate, in the presence of up to 100 mg/ml kanamycin, and bud regeneration from transformed roots occurred in the presence of up to 30 mg/ml kanamycin. Callus and buds from control (non-transformed) plants failed to proliferate or regenerate, respectively, in the presence of

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kanamycin at concentrations above 10 mg/ml. Ninety-four independent clones resulting from different transformation events have been established, of which 52 are phenotypically true-to-type.

*P. halepensis* embryos, seedlings and mature buds were also transformed using an *A. rhizogenes* strain, as monitored by root formation and/or GUS expression. More than 85 % of the mature embryos show GUS expression in the radicle and only 24 % in the cotyledons.

*Selected publications:*

Nadel, B.L., A. Altman, S. Pleban and A. Huttermann (1991). ***In-vitro* development of mature *Fagus sylvatica* L. buds. I. The effect of medium and plant regulators on bud growth and protein profiles.** J. Plant Physiol. 138: 596-601.

Nadel, B.L., A. Altman, S. Pleban, R. Kocks and A. Huttermann (1991). ***In vitro* development of mature *Fagus sylvatica* L. buds. II. Seasonal changes in the response to plant growth regulators.** J. Plant Physiol. 138:136-141.

Nadel, B.L., G. Hazan, R. David, A. Altman and A. Huttermann (1992). ***In vitro* propagation of *Populus* species: responses to growth regulators and medium composition.** Acta Horticulturae 314: 61-68.

Altman, A., A. Ya'ari, D. Pelah, A. Gal, T. Tzfira, W-X. Wang, O. Shoseyov, A. Vainstein and J. Rivov (1995). ***In vitro* organogenesis, transformation and expression of drought-related proteins in forest tree cultures.** In: M. Terzi et al., eds., Current Issues in Plant Molecular and Cellular Biology, Kluwer Academic Publ., pp. 87-94.

Tzfira, T., Ben-Meir, H., Vainstein, A., and Altman, A. (1996). **Highly efficient transformation and regeneration of aspen plants through shoot-bud formation in root cultures.** Plant Cell Rep. 15: 566-571.

Altman, A., D. Pelah, O. Yarnitsky, T. Tzfira, A. Ya'ari, W-X. Wang, O. Shoseyov, A. Vainstein, A. Huttermann and S. Wang (1996). **Towards water stress-tolerant poplar and pine trees: molecular biology, transformation and regeneration.** In: A.R. Ahuja, ed., Somatic Cell Genetics and Molecular Genetics of Trees, Kluwer Press (in press).

Tzfira, T., O. Yarnitzky, A. Vainstein and A. Altman (1996). **Highly efficient transformation and regeneration of transgenic aspen plants through shoot-bud formation in**

Inoculum injection into intact seedling hypocotyls induced callus and root formation in 64 % of the seedlings. In addition, adventitious roots and root primordia regenerated in 74 % of the transformed shoots, following transformation of cytokinin-induced brachyblasts (see above). PCR and Southern blot analysis confirmed the presence of rolC and rolB genes and the uidA-transgenic nature of the regenerated plants and tissues, respectively, of *Populus tremula* and *Pinus halepensis*.

**root culture, and transformation of *Pinus halepensis*.** In: A.R. Ahuja, ed., Somatic Cell Genetics and Molecular Genetics of Trees, Kluwer Press (in press).

Tzfira T., O. Yarnitzky, A. Vainstein and A. Altman (1996). **Transformation and regeneration of *Pinus halepensis*.** Plant Cell Rep. (in press).

Tzfira, T., C.S. Jensen, A. Vainstein and A. Altman (1996). **Aspen transformation procedures: oncogenic *Agrobacterium rhizogenes* versus disarmed *Agrobacterium tumefaciens*.** In: A. Altman and M. Ziv, eds, Acta Horticulturae (in press).

Tzfira, T., A. Vainstein and A. Altman (1996). **Improved rooting ability and root system performance in transgenic aspen plants.** In: A. Altman and Y. Waisel, eds. The Biology of Root Formation and Development (in press).

Pelah, D., W. Wang, A. Altman, O. Shoseyov and D. Bartels (1996). **Differential accumulation of water-stress related proteins, sucrose synthase and soluble sugars in *Populus* genotypes which differ in their water-stress response.** Physiol. Plant. (in press).

Tzfira, T. et al. (1996). **A simple computer-image-analysis technique for measuring root surface area** (submitted).

Pelah, D., O. Shoseyov, A. Altman and D. Bartels (1996). **Water-stress response in aspen (*Populus tremula*): differential accumulation of dehydrin, sucrose synthase, GAPDH homologues and soluble sugars** (submitted).

Pelah, D. O. Shoseyov and A. Altman (1996). **Further characterization of BspA, a water-stress responsive boiling stable protein in aspen (*Populus tremula*) and cloning of the BspA gene** (submitted).





## Calendar of Meetings and Workshops

### 12-16 January 1997, International Plant and Animal Genome V Conference.

Town and Country Hotel, San Diego, CA. The meeting will offer satellite sessions and workshops. Organizers: Scherago International, Inc. Email: pag5@scherago.com or check out website <http://probe.nalusda.gov:8000>. On site registration fee is \$375.00. Meeting includes the Forest Tree Genome Workshop on January 12, 1997 organized by Toby Bradshaw. Email [toby@u.washington.edu](mailto:toby@u.washington.edu).

**9-12 June 1997, 24th Biennial Southern Forest Tree Improvement Conference.** Orlando, Florida. Organizers: University of Florida. Preregistration is \$180.00 before April 30, 1997 and \$250.00 after. Papers and posters to include all aspects of forest genetics, tree improvement and biotechnology. Invited papers on fast growing exotic tree species are encouraged from around the world. To register, call (352) 392-5930 or write 24th SFTIC, IFAS Office of Conferences, University of Florida, PO Box 110750, Gainesville, FL 32611 or visit website <http://www.ifas.ufl.edu/~conferweb/sftic.htm>.

**29-1 July-August 1997, Forest Tree Genome Workshop.** Institute of Forest Genetics, Placerville, CA. A four day hands-on workshop covering molecular markers, genetic diversity, genetic mapping, genome organization, QTL mapping, and marker assisted breeding and gene sequencing. Contact: David Neale at (510) 559-6436 or email: [dbn@s27w007.pswfs.gov](mailto:dbn@s27w007.pswfs.gov).

**5-7 August 1997, Western Forest Genetics Association (WFGA).** University of California, Berkeley, CA. Contact: Tom Conkle at (510)559-6421 or email: [mtc@s27w007.pswfs.gov](mailto:mtc@s27w007.pswfs.gov).

**13-16 August 1997, Joint Meeting of the IUFRO Working Parties 5.04-07 and 5.04-06 Somatic Cell Genetics and Molecular Genetics of Trees.** Loews Le Concorde Hotel in Quebec City, Canada. The themes of the meeting are: forest tree tissue culture, genetic transformation and engineering of trees, gene isolation and expression, and molecular marker assisted tree improvement. Registration fee is \$200 Canadian. For further mailings about the conference contact: Pierre J. Charest, Canadian Forest Service, Natural Resources Canada, 580 Booth Street, 7th floor Ottawa, Ontario, Canada, K1A 0E4, tel: (613) 947-9011, email: [pcharest@am.nrcforestry.ca](mailto:pcharest@am.nrcforestry.ca) or Armand Sequin, Canadian Forest Service, Natural Resources Canada, 1055 du PEPS -PO Box 3800, Saint-Foy, Quebec, Canada, Tel: (418) 648-5832, email: [Sequin@cfl.forestry.ca](mailto:Sequin@cfl.forestry.ca).

**18-21 August 1997, International Workshop on Wood Quality at the 26th Biannual Meeting of the Canadian Tree Improvement Association.** Quebec City, Canada. Contact: Dr. Tony Zhang, Resource Assessment and Utilization Group, Forintek Canada Corp. 319, rue Franquet, Sainte-Foy, Quebec, Canada G1P 4R4, tel: (418) 659-2647, email: [tony.zhang@gc.forintek.ca](mailto:tony.zhang@gc.forintek.ca).

**24-29 August 1997, IUFRO Conference on Silviculture and Improvement of Eucalypts.** Salvador, State of Bahia, Brazil. Conference topics include tree improvement, biotechnology silviculture, and environmental and social impacts of Eucalypts. Contact: EMBRAPA-Florestas, CP 319, CEP 83411-000, Colombo, Brazil, email: [eucalypt@cnpf.embrapa.br](mailto:eucalypt@cnpf.embrapa.br).

**21-27 September 1997, 5th International Congress of Plant Molecular Biology.** Singapore. Conference on all areas of plant molecular biology/biotechnology, including those relevant to the agricultural problems of developing nations. Contact: Congress Secretary, 5th International Congress of Plant Molecular Biology, Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602-7229, fax (1) 706-542-2090, email : [ldure@uga.cc.uga.edu](mailto:ldure@uga.cc.uga.edu).

**12-17 October 1997, Diversity and Adaptation in Oak Species.** State College, PA. This is the second meeting of the International Union of Forestry Research Organizations Working Party on Genetics of *Quercus* (2.08.05). Contact Dr. Kim Steiner at tel: (814) 865-9351, fax: (814) 865-3725, email: [kcs@psu.edu](mailto:kcs@psu.edu).

**1-4 December 1997, NZ FRI-IUFRO Conference on the "Genetics of Radiata Pine".** Rotorua, New Zealand. Contact: 1997 NZ FRI-IUFRO Conference, NZ Forest Research Institute, Private Bag 3020, Rotorua, New Zealand, Tel: 64 7 3475899, fax: 64 7 3479380, email: [iufro97@fri.cri.nz](mailto:iufro97@fri.cri.nz).

**Meeting Report: Joint Meeting of IUFRO Research Groups on Population, Ecological and Conservation Genetics (S2.04-01) and Genetic Aspects of Air Pollution and Climate Change (P2.05-05), University of British Columbia, Canada, August 5-9, 1996**

**Yong-Bi Fu and John E. Carlson, Department of Forest Sciences, UBC, Vancouver, Canada V6T 1Z4**

More than 100 people from 27 countries converged on the campus of University of British Columbia (UBC), Vancouver, Canada, to participate in the international symposium, "Diversity and Adaptation in Forest Ecosystems in A Changing World", held in early August by the International Union of Forest Research Organizations (IUFRO) Research Groups S2.04-01 and P2.05-05. This symposium was organized by Francis Yeh (University of Alberta, Canada) and John Carlson (UBC, Canada), and sponsored by the Canadian Forest Service (Natural Resources Canada), Department of Forest Sciences at UBC, the Centre for International Forestry, the International Plant Genetics Resource Institute, and the B.C. Ministry of Forests.

The meeting featured three general sessions on adaptation and adaptability, inference on population differentiation, and effects of disturbances on diversity in forest ecosystems, complemented by a vigorous, open group discussion on the last day. In addition to 13 invited papers, 16 voluntary papers, and more than 40 posters covering a wide range of topics, there was also a demonstration of two new computer programs (POPGENE by Francis Yeh and ECOGENE by Bernd Degen). Participants enjoyed a field trip to Victoria and the Cowichan Lake Research Station, ending with a tasty barbecue hosted by the B.C. Ministry of Forests. What follows is a brief review of some of the highlights in this symposium from our own perspectives.

In his keynote speech, Gene Namkoong (UBC, Canada) outlined the linkages between diversity and adaptation that serve as a basis for most conservation programs. From a theoretical perspective, Gene emphasized the dynamic nature of population fitness in varying environments, raised several technical issues toward understanding dynamic systems, and presented arguments, for example, concerning the value of reductionism in understanding genetics of adaptation in a dynamic system as a whole. In contrast, the following day David Neale (USDA Forest Service) presented examples based on an experimental approach, mapping genes of large effects controlling adaptive traits such as budflush and budset in Douglas-fir, to demonstrate that a better understanding of the genetics of adaptation, in terms of gene numbers and effects, can be gained using the gene mapping approach, while pointing out that gene mapping alone will not be sufficient for completely understanding dynamic systems.

Many presentations and posters demonstrated the value of genetic markers in clone identification, mating systems and paternity analyses, evaluation of genetic variation within and among populations, studies of adaptive traits, detection of quantitative trait loci, and genome mapping. The many types of genetic markers available, including terpene polymorphism, immunological proteins, allozymes, RAPDs, SSRs, AFLPs, RFLPs, cDNAs, minisatellites, and

microsatellites, were all mentioned. In addition to David Neale's presentation on QTL research, there were several interesting papers representing some new applications of genetic markers worthy of mention. Jean Beaulieu (Natural Resources Canada) and his colleagues showed that two populations of spruce from central Quebec with low observed heterozygosities at enzyme loci did not show decreased levels of genetic diversity at anonymous polymorphic DNA loci. Yong-Bi Fu (UBC, Canada) and his colleagues showed a new use of RAPDs to infer paternal success in a spruce seed orchard based on a similarity index approach. Bonnie Furman (NC State, USA) and her colleagues illustrated the application of phylogenetically informative RAPD markers to aid conservation of central American and Mexican pines. Mr. Dayanandan (Univ. of Massachusetts, USA) and his coworkers identified several microsatellite markers useful for population and conservation genetics of tropical trees.

At the end of the conference, Gerhard Muller-Stark bravely attempted to arrive at a democratic census on a single genetic marker system that the forest genetics community should use to enhance direct comparison of results and maximize information obtained. However, the only consensus was

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that no consensus is possible at this time as each marker system has advantages and limitations and that it is best left to the individual researcher to determine the suitability of genetic markers in various applications and situations.

Genetic differentiation of populations was among the more prevalent topics in the symposium. Antoine Kremer (INRA, France) introduced a composite, multilocus measure of population differentiation in forest trees which accounts for allelic associations by combining both gametic and non-gametic effects. He also showed that discrepancy in within and between matrices of correlation of allelic frequencies is a major cause of multilocus population differentiation. Kermit Ritland (UBC, Canada) presented a method for estimating pairwise gene flow and individual population divergence. It is based on inbreeding coefficients of each population and the relatedness of two populations, both relative to an outgroup (such as a geographically separated population). While this method should be useful in evaluating the extent of population fragmentation and mixing caused by forest practices, it requires more informative markers like microsatellites. Outi Savolainen (University of Oulu, Finland) presented some interesting evidence for differentiation resulting from random genetic drift, colonization history, and natural selection, and discussed implications of these differentiation patterns for genetic conservation. Yoshihiko Tsumura and his colleagues (Forestry & Forest Products Research Institute, Japan) showed that there was strong population differentiation and very limited within-population variation for mitochondrial DNA in Japanese beech. Valery Putenikhin (Russian

Acad. Sci., Ufa) discussed population differentiation of Siberian spruce and Scots pine in the South Urals. Fiorella Villani (CNR, Italy) and her colleagues presented interesting evidence for gene flow and divergence in Chestnut from Turkey. On a larger scale, Remy Petit (INRA, France) applied mtDNA and cpDNA markers to infer phylogeography, recolonization, and migration routes in European oaks and beech, as well as in the argan tree of southern Morocco.

Gerhard Muller-Starck (University of Munich, Germany) demonstrated a case study for genetic response of European beech populations to environmental stress. He showed that, under increasing complexity of stress conditions, heterozygosities tend to be larger and genetic diversity increases in tolerant samples, and that complex and long lasting environmental stress seems to result in complex genetic response at the multi-locus level and genetically diverse individuals obviously show a viability advantage under complex environmental stress. He also stressed that ecological genetics should continue to play a major role in understanding the adaptation and dynamics of forest populations. Florian Scholz (Institute of Forest Genetics, Germany) profiled his group's research on genetic effects of pollution, in terms of viability, genetic drift, mating systems, gene flow, and mutation. Jochen Kleinschmidt (Lower Saxony Forest Research Institute, Germany) discussed the consequences of air pollution and climate changes for managing genetic resources and concluded that maintenance and increase of adaptability become more urgent under the auspices of air pollution and climate change.

Conservation policy is another topic that received great attention and extensive discussion. Dennis Joyce and Brad Graham (Ontario Forest Research Institute, Canada) introduced Ontario's genetic resources management framework and implementation based on principles from landscape ecology, conservation biology, plant geography, quantitative genetics and population genetics. Population size, number and distribution of subpopulations within individual genetic resource management units are used as the primary indicators of vulnerability to genetic erosion. The principles associated with effective population size and metapopulation dynamics were used to develop guidelines for managing genetic drift and inbreeding. In the conceptual framework, there is still a lack of active breeding approaches for incorporating long-term forest genetic conservation, such as Namkoong's multiple population breeding strategy. Ilker Acar (Ege Forest Research Institute, Turkey) demonstrated a project on *in-situ* conservation of genetic diversity and its application in Turkey. Tim Boyle (CIFOR, Canada) discussed the genetic effects of various harvesting procedures in tropical forests and emphasized the need for multidisciplinary research, including assessment of social-economic benefits. Om Rajora (University of Alberta, Canada) outlined all the possible factors from forest practices that may influence genetic diversity and their implications for sustainable forest management and gene conservation. Despite these discussions, it remains less than clear what the role of research in genetics and ecology are in conservation policy making.

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Csaba Matyas (University Sopron, Hungary) revisited provenance tests for assessing major trends of geographic variation in adaptive traits and suggested cautions in interpreting adaptation. Fan Kung (Southern Illinois University, USA) demonstrated geographical variation patterns of adaptability of white oak provenances and black walnut provenances. Eduardo Notivol (SIA-DGA, Spain) illustrated the variation and adaptation of Scots pine provenances in Spain. Xihuan Shen (Beijing Forestry University, China) showed the variations in adaptation associated with climate change for major conifer tree species in Northern China. These talks demonstrated that common garden tests still play an important role in providing information on adaptation and genotype-environment interactions.

The symposium ended with the expression of concern about the great unknowns and uncertainty remaining in diversity, adaptation, and forest conservation. The participants unanimously agreed that their next meeting should be held in 3 years at the University of Munich and should expand on the theme of adaptation and conservation by meeting together with IUFRO forest ecology research groups.

For the abstracts of invited papers, voluntary papers and posters, please refer to the internet at "dendrome@s27w007.pswfs.gov". The thirteen invited papers will be published in book format by December, 1997.

## Meeting Report, Southern Research Information and Exchange Group, "Novel Applications of Molecular Markers in Forest Trees"

Houston, Texas, June 23-26, 1996

Claire Williams, Texas A&M University and  
Jerry Tuskan, Oak Ridge National Lab, Department of Energy

The 1996 SRIEG Conference "Novel Applications of Molecular Markers in Forest Trees" was held at Texas A&M University's Institute of Biosciences and Technology in Houston, Texas from 23-26 June. The 103 attendees included international visitors from 12 countries, graduate students, postdoctoral scientists, emeriti and researchers from companies, universities and government institutes. The proceedings of the workshop are published only via the World Wide Web: <http://mslisma.tamu.edu/staff/tom/SRIEG/srieghome.html>.

The SRIEG-40 meetings have been organized traditionally for exchanging valuable research information among tree breeders in the southern United States. A topic is chosen and experts are invited to speak. We altered the 1996 meeting format in two ways: 1) the program was designed to foster collaboration among international breeders, molecular biologists, quantitative and population geneticists, and 2) a poster session was added to encourage attendance and scientific recognition of international visitors, graduate students and other young scientists. Funds from our sponsors enabled wider participation and numerous poster awards: Department of Energy, USDA-Plant Genome Program, Texas A&M University, Weyerhaeuser Foundation and Westvaco Corporation.

June 24: The first session emphasized genome mapping basics: how unique features of conifer genome architecture affect mapping (C. Kinlaw and K. Hutchison), progress and utility of PCR-codominant marker types (C. Echt, D. Harry and D. Nelson) and framework map construction (B. Liu). The second session addressed moving from QTL to physical mapping (D. Neale), integrating genetic and cytological maps (R. Doudrick), unique applications of cytoplasmic DNA markers (W. Powell et al.) and questionable trans-gene stability in woody plants (R. Ahuja). Our keynote speaker was Dr. Robert Merrifield, emeritus director of the Texas Agricultural Experiments Station at Texas A&M University and longtime SRIEG-40 sponsor. Dr. Merrifield summarized the national status of agricultural research funds, emphasizing the strong movement towards reduced funding for agricultural research in the U.S.

June 25: This session focused on breeders' applications of marker technology. The breadth of application and species was impressive: finding gender-specific markers in the dioecious willows (J. Tuskan, U. Gullberg and C. Allstrom), marker-assisted selection in loblolly pine (T. Byram, S. McKeand and D. O'Malley), QTL validation in

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eucalyptus (M. Byrne and D. Grattapaglia), gene conservation needs in Central America and Mexico (B. Dvorak, S. Carson). The afternoon session expanded on this topic further with the case study for Populus, the “laboratory mouse of forest trees” (T. Bradshaw, B. Stanton, M. Stine and B. Li), marker-aided selection for Douglas-fir (S. Strauss, R. Johnson, N. Wheeler, K. Krutovskii and C. Dean) and QTL mapping strategies for radiata pine (J. Chaparro et al.). Our keynote speaker was Dr. Charles Stuber, USDA-ARS, who spoke about the genetic basis of heterosis and successful molecular-aided selection in maize “enhanced” inbred lines.

June 26: The last session emphasized research applications of genome mapping. The first two talks addressed

how coancestry and genetic load could be dissected in eucalypts (B. Potts, N. Borralho, C. Hardner and R. Vaillancourt) and in loblolly pine (C. Williams). The lack of fitness in some allozyme genotypes in Douglas-fir was a novel application of markers (B. Bongarten and N. Wheeler) and population genetics has benefited greatly with the advent of genomic mapping technology (D. O’Malley). The last talk of the session (K. Hutchison, M. Greenwood, O. Johnson and T. Skroppa) showed how genetic after effects of environmental stress in conifers influence where seed orchards should be placed in boreal zones. The keynote speaker was Dr. Andrew Paterson, Texas A&M University, who spoke about applications of genomic mapping to crop evolution and comparative QTL analysis research.



**Meeting Report, Western Forest Genetics Association Annual Meeting**

**Newport, Oregon July 29 - August 1, 1996**

**Sally Aitken and Tom Adams**

The 1996 Western Forest Genetics Association (WFGA) was held from July 29th through August 1st in Newport, Oregon, on the cool and windy, yet very scenic, Oregon coast. The theme of the meeting was “Genetics of Adaptation”. The meeting was organized by forest geneticists at Oregon State University, the USDA Forest Service PNW Research Station, and the Oregon Department of Forestry. Ninety delegates from the United States, Canada, Finland, Denmark, Sweden, Ghana and New Zealand participated in this meeting. The agenda included one day of satellite meetings (the North American Quantitative Forest Genetics Workshop and the Pacific Northwest Tree Improvement Cooperative Annual Meeting), two days of invited and volunteer presentations, and a one-day field trip north along the Oregon coast.

The North American Quantitative Forest Genetic Workshop, chaired by Randy Johnson, focussed on “Maintaining genetic variation in future generations”. The discussion included considerations for maintaining current levels of genetic diversity in natural populations as well as maintaining adequate levels

of variation in breeding populations. Michael Lynch from the University of Oregon discussed minimum population sizes needed to maintain genetic variation in natural populations, given rates of mutation for quantitative traits. He suggested that effective population sizes in the low thousands would be necessary to maintain a balance between variation arising through mutation and being lost through drift and weak selection. Gene Namkoong discussed the use of multiple population breeding strategies to maintain and even increase genetic variation in breeding programs. Bill Libby raised the question of coadapted gene complexes and our poor understanding of this issue and how it relates to tree improvement. Steve McKeand and Bill Lowe outlined breeding strategies for maintaining genetic variation in loblolly pine in breeding programs in North Carolina and Texas, respectively.

The theme of the WFGA Annual Meeting was “Genetics of Adaptation”. This theme was chosen due to the growing body of knowledge about the physiological and molecular genetics of adaptive traits, increasing interest in predicting genetic response of populations to changing climates, and the trend towards incorporating adaptive as well as economic traits in breeding programs. The program committee, chaired by Bart Thielges of Oregon State University, invited a mix of speakers from both within and outside the forest genetics community to discuss patterns of adaptation in natural populations and the issue of maintaining adaptability in breeding programs. The quality and quantity of volunteer papers and poster presentations provided further evidence of the degree of interest in this theme.

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The meeting opened with a day focusing on patterns of adaptation in natural populations. Jim Ehleringer, an ecophysiologicalist from the University of Utah, was invited to talk about adaptation in conifers in aridland ecosystems on the Colorado Plateau. He discussed research using stable isotopes of hydrogen, oxygen and carbon to better understand resource use in these systems. Perhaps the most surprising result was evidence of strong geographic differences within species in the use of deep water from winter rains versus shallower moisture from summer rains. It appears that some trees in certain arid areas do not utilize summer precipitation, even when it is readily available. This begs the question of the function of shallow roots in these areas, which may be primarily for nutrient rather than water absorption.

With growing interest in and understanding of both adaptive physiology and molecular genetics in trees, the tools are now available to explore the molecular basis for adaptive traits. David Neale of the Institute of Forest Genetics in California gave an invited talk on this subject. Quantitative genetic and breeding theory is based on the polygenic model of inheritance, assuming traits are controlled by a large number of genes with small, additive effects. David summarized experimental evidence that adaptive traits may typically be under oligogenic control, with a small number of genetic loci having larger and not necessarily additive effects. If traits are under oligogenic control, quantitative trait loci (QTLs) can be identified and mapped. He presented preliminary results from a project to map QTLs controlling

bud phenology in Douglas-fir as an example of this area of research.

Frank Sorensen of the US Forest Service Pacific Northwest Research Station in Corvallis has been investigating patterns of adaptive variation in Pacific Northwest conifers for many years. He was invited to summarize his wealth of knowledge on this subject at the meeting. Frank's presentation first emphasized the large amount of within-population genetic variation in most conifer species, resulting in part from gene flow via pollen and seed dispersal, and providing the raw material for the development of adaptive patterns. He then provided examples comparing and contrasting patterns of adaptive variation with respect to climatic and geographic variables, and discussed the possible reasons for differences among species including historical factors, differences in patterns among traits, and varying plasticity of response of different species.

A field trip up the Oregon Coast (on a rare, sunny day) provided examples of adaptive problems in coastal Douglas-fir. A visit to the Hebo test site of the 83-year-old Heredity Study established by forest genetics pioneer Thornton T. Munger, consisting of progeny of a total of 120 parent trees from 13 locations in Washington and Oregon. The design of the test makes it difficult to analyze statistically, but a visit to the site provides some strong messages. The primary conclusions from this study are that: 1) under-stocking is the primary symptom of maladaptation; 2) inherent growth rates are stable over time; and 3) yields are initially related to growth rate, but become increasingly related to survival. This last point is very important as some provenances that had good growth rates initially (for the first 20 years)

suffered considerable damage and mortality from some extreme climatic events such as the November 1956 freeze that damaged and killed some mature trees.

A current issue of considerable concern for forestry in coastal Oregon is the recent increase in young Douglas-fir stands infected with Swiss Needle Cast, an endemic disease which causes premature needle drop and reduced growth rates. This issue was discussed at a stop on the field trip. The problem primarily appears to be the planting of pure Douglas-fir stands in the very wet maritime environment along the coast that historically supported stands of Sitka spruce and western hemlock with only a minor component of Douglas-fir. There is also evidence that heavy infection can result from the use of seed collected from areas east of the coastal fog belt. Randy Johnson reported on significant within-population genetic variation for rates of needle yellowing and loss, but surprisingly there is no genetic correlation between rate of defoliation and growth rate. A substantial effort is underway to identify cultural treatments, primarily fertilization, to try to invigorate heavily infected stands of 10- to 20-year-old trees.

The second day of papers focused on "Maintaining Adaptability Under Domestication". Warren Kronstad, a wheat breeder from Oregon State University, gave an invited talk on breeding strategies in self-pollinated crop species to achieve and maintain adaptability. His presentation was a wake-up call for all breeders regarding the adaptive problems that can arise with breeding programs based on a narrow genetic base. Warren

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presented an overview of wheat breeding, and the current race to maintain or improve grain quality and quantity, while staying ahead of the many types of rust that can devastate crops. As hybrid wheat cultivars become near-homozygous lines after five generations of selfing, strategies for maintaining adaptability are important. In recent years, wheat yields have levelled out while worldwide wheat consumption has risen. Diseases overcome genetic resistance in new cultivars in just a few years. Wheat breeders are now employing a variety of strategies to develop broad adaptability including testing and selection over a wider range of environments, deployment of cultivar mixtures rather than planting isogenic crops, and developing hybrid cultivars.

After hearing of the woes of wheat breeding, the focus turned to maintaining adaptability in tree breeding populations. Reinhart Stettler drew on his extensive research experience to discuss the role of adaptability in poplar culture. He addressed issues of phenotypic plasticity, developmental homeostasis and yield stability with respect to adaptability in poplar species. *Populus trichocarpa* was used as an example to discuss morphological, anatomical and physiological mechanisms of adaptability in poplars. *P. trichocarpa* exhibits a high level of phenotypic plasticity that plays an important role in adaptation to native poplar habitats. This plasticity can be further enhanced through hybridization of *P. trichocarpa* with *P. deltoides* and subsequent clonal selection. Adaptability can be increased through deployment strategies of selected clones at the stand level.

The final invited speaker of the meeting was Cheryl Talbert of

Weyerhaeuser Company, who discussed strategies for maintaining adaptive performance in advanced-generation populations of coastal Douglas-fir. She discussed a two-part strategy for maintaining adaptability in Douglas-fir. First, all selected families are screened for adaptive traits related to response to environmental stresses. Second, the response of specific genotypes to major operational environment types in both research trials and operational plantations is monitored. This strategy requires the close cooperation of breeders and foresters. A thorough understanding of the adaptive responses of specific genotypes to specific environments will, in the long term, lead to a more efficient deployment of improved materials into the environments to which they are best suited.

In addition to the invited presentations summarized here, volunteer paper and poster presentations covered a wide range of topics relating to the meeting theme. A common subject area was the assessment of genetic variation for physiological traits related to adaptation to environmental stresses, including cold hardiness and phenology; drought response related traits including water-use efficiency, hydraulic architecture and vulnerability to embolism of xylem water columns; gas exchange; and disease resistance. Several presentations described gene conservation programs. There were also a number of reports on population genetics projects.

The 1997 Western Forest Genetics Meeting will be held in Berkeley, California on August 4-7 and will be hosted by the Institute of Forest Genetics. Contact Tom Conkle (mtc@s27w007.pswfs.gov) for further information.

## Meeting Report: International Symposium on 'In Situ Conservation of Plant Genetic Diversity'

Zeki Kaya, Department of Biological Sciences, Middle East Technical University 06531 Ankara, Turkey (zeki-kaya@metu.edu.tr)

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Turkey is one of the domestication centers for crop species and the starting place for ancient agriculture in the World. Thus, an international project, *In situ Conservation of Genetic Diversity in Turkey*, was initiated in 1993 in Turkey. The project is being supported by the Global Environmental Facility (GEF) and conducted in collaboration with the Ministries of Agriculture and Rural Affairs, Forestry, and Environment of the Republic of Turkey. The goal of the project is to develop *in situ* gene conservation programs for target plant species selected from wild relatives of crop, fruit tree and globally important forest tree species in selected pilot sites. These sites are Ceylanpinar State Farm and Kaz Mountain which have remarkable importance in terms of genetic resources for many economically important cultivated plant species and richness in endemic plant species, respectively.

Project goals are also at training and educating of researchers in the field, institutional strengthening of involved agencies, developing public awareness programs on *in situ* conservation, as well as preparing a *National Plan on in situ conservation of plant genetic diversity in Turkey* based on experiences gained from the project. Since the project is the first of its kind in the

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world, the Scientific Advisory Committee (SAC) of the project recommended that an international symposium should be held on *in situ* conservation of plant genetic diversity to share the experiences from the project with other countries as well as get feed back from an international audience on the *National Plan*. The international symposium was held on November 4-8, 1996, in a beautiful resort hotel, the Adora Golf Resort Hotel in Belek, Antalya. The Adora Golf Resort Hotel is located on one of the finest beaches of the Mediterranean Coast in Southern Turkey and offered many facilities for leisure time activities such as tennis, indoor and outdoor swimming pools, beaches, golf, surfing, sea parachuting etc. The symposium was organized by the Ministries of Agriculture and Rural Affairs, Forestry, and Environment of the Republic of Turkey, The SAC and The World Bank. For some, especially for SAC members, this symposium started early and ended late since the annual SAC meeting was also held in the same place where the symposium was held.

On the first day of the meeting ( Nov. 4, 1996), there were three sessions. These were an *Inaugural Session* on "Exploitation of plant genetic resources", *Session 1* on "Current Status of Genetic Diversity in the World, and *Session 2* on "*In situ* Conservation of Genetic Diversity Project". The keynote speaker for the *Inaugural Session* was to be J.R. Harlan, but due to health problems, he was unable to attend the symposium. The opening presentation was then given by S. L. Krugman (USDA, Forest Service). He stressed the importance of *in situ* conservation of plant genetic resources in the world and explained the difficulties of *in situ* conservation with examples of other international projects funded by the World Bank. In this session, the second speaker was N. Durutan who is the World Bank task manager for the *In situ Conservation of Genetic Diversity project in Turkey*, she reviewed the GEF projects which were funded by the World Bank, with specific emphasis given to Turkish GEF projects. In *Session 1* of the symposium, the first speaker was J. G. Hawkes of University of Birmingham, UK. He summarized conservation activities mainly in crop species in the world and pointed out the value of *in situ* conservation for crop species where the evolutionary processes are allowed to continue. He also stated that the lack of this dynamic process is one of the major problems in *ex situ* conservation programs. The second speaker of the session was A. Tan of Aegean Agricultural Research Institute, Izmir, Turkey and she summarized the current status of plant genetic resources and conservation activities, with special emphasis on wild

relatives of crop species. The third speaker of the session was Z. Kaya of Middle East Technical University, Ankara. He presented an overview of the current status and problems of forest genetic resources and conservation activities in Turkey. He stressed the importance of incorporation of conservation concepts into regular forestry practices carried out by the Turkish Forest Service. *Session 2* of the symposium was dedicated to the introduction of Turkish project -*In situ Conservation of Genetic Diversity in Turkey* to the international audience. The session began with the talk by Y. Anikster of Tel Aviv University, Israel. He shared the Israeli experience with *in situ* conservation of wild wheat in Israel. This was followed by the speakers from the Turkish Ministry of Agriculture and Rural Affairs, and Ministry of Forestry. They presented the pilot project on *In situ Conservation of Genetic Diversity in Turkey* and shared experiences and the progresses made in the project with international participants.

On November 5, 1996 two sessions were held. The first (*Session 3*) was on "*Ecogeography and distribution of plant and forest tree species*" and the second (*Session 4*) was on "*Genetic diversity of plant and forest tree species*". The first speaker of *Session 3*, N. Maxted (University of Birmingham, UK), talked about the role of ecogeographic techniques in *in situ* conservation of plant genetic resources. He demonstrated this with data gathered in the legume genus *Vicia*. The second speaker of *Session 3* was F. Yaltirik (University of Istanbul, Turkey) who informed the participants on forest types and important tree species of Turkey in terms of *in situ* conservation programs. The first speaker of *Session 4* was M. Feldman of Weizman Institute of Science, Israel. He outlined the procedures and approaches to study genetic diversity in wild relatives of crop species. Factors affecting genetic diversity in wild relatives of several major crop species in different habitats were discussed as in relation to *in situ* conservation. The second speaker of the *Session 4* was F. T. Ledig (University of California, Davis and USDA- Institute of Forest Genetics, Placerville, California). In his keynote address, he reviewed the genetic diversity levels in woody plant species and discussed the factors leading to the high genetic diversity levels in conifers. He completed his talk with the Mexican spruce example in which population sizes have been severely reduced. He pointed out that without interference such as helping gene flow between disjunct populations, *in situ* conservation will fail to conserve genetic resources of this species in Mexico.

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November 6, 1996 was the field trip day. Symposium participants were anxious to see the countryside after hearing two days of technical sessions. The field trip was organized by the Ministry of Forestry and the guide was H. Usta from the Southwest Mediterranean Forest Research Institute, Antalya. The trip started with a visit to the Koprulu Canyon National Park which is rich in endemic plant species and history. The well-preserved *Cupressus sempervirens* var. *horizontalis* stands were impressive. The view of the Toros Mountains and conglomerate stone formations were breath taking. The field trip guide, H. Usta, had considerable knowledge of Turkish forestry, but was also an excellent tourist guide. His explanations on the history of Aspendos was very informative. The Aspendos, which is one of the best preserved ancient amphitheatres in the world, was the last stop of the field trip.

On November 7, 1996 the symposium continued with the speakers of *Session 4*. The third speaker of *Session 4*, D. B. Neale (USDA- Institute of Forest Genetics, Placerville, California & University of California, Davis) discussed the estimation of genetic diversity within tree populations in terms of neutral vs. adaptive genetic variation. Based on the results of studies conducted in his lab in relation to genetic mapping of quantitative traits, he emphasized that the future gene conservation (*in situ* as well as *ex situ*) programs should attempt to conserve adaptive genes with help of newly developed DNA markers. *Session 5* of the symposium was about management systems for *in situ* conservation of plant species. The first speaker of this session was O. Bakir of Ankara University, Ankara. He focused on the possible management practices in the Gene Management Zones specifically set up for pasture plant species.

The afternoon sessions of November 7, 1996 were reserved for a discussion of the draft "*National Plan for in situ Conservation of Plant Genetic Diversity in Turkey*" prepared by Z. Kaya, E. Kun (Ankara University) and A. Guner (Abant I.B. University, Bolu, Turkey). First, the draft plan was briefly presented by the above authors and their expectations from the international participants on the draft plan were stated. The afternoon discussion was moderated by W. T. Adams from Oregon State University, Corvallis. W. T. Adams organized the discussion so that most of the

time could be used to get the comments and views of the symposium participants on the draft Turkish National Plan.

November 8, 1996 was the last day of the symposium. The second speaker of *Session 5*, C.I. Millar from USDA- Institute of Forest Genetics, Placerville, CA, pointed out that a single approach such as gene management zones (GMZ) should not be considered as the only option in *in situ* gene conservation of forest trees. Other options should be also utilized depending on the goals of *in situ* gene conservation and strategies for effectiveness of *in situ* conservation programs. The other options were discussed with the examples from California *in situ* conservation programs. The final session (*Session 6*) of the symposium was on the "*Data Management Systems for in situ Conservation*". The speaker was E. D. Hunt from ESRI, Redlands, CA. He presented an environmental data base model based on Geographical Information System (GIS) that meets the needs of *in situ* genetic conservation for geographic data. There were also numerous voluntary and poster presentations in each session of the symposium. They provided valuable information and experiences on *in situ* conservation of wild relatives of crop species and globally important forest tree species in Turkey as well as in the world. Selected symposium papers will be published in a proceedings in the future. The symposium was a success in terms of introducing the Turkish *in situ* gene conservation project and the Turkish *National Plan* to the international audience as well as an exchange of information and experiences in *in situ* genetic conservation among the participants.



## From the Editor

The Dendrome Project is now old enough to experience its first change in staff. Bradley K. Sherman came to the project in November 1991 to work with me to design and develop the newly established genome database project. Brad was the perfect person for the job, although I must admit that I did not fully appreciate this at the time I hired him. Brad was an experienced programmer and user of the Unix operating system. He quickly made hardware purchases and got our server up and running. One of Brad's greatest talents was his knowledge of the Internet. At a time when most of us in biology had barely heard of the net or even used email, Brad was already a master of the net. In today's environment it is taken for granted that any database manager would be fully knowledgeable of the Internet, but in 1991 Brad was way ahead of the curve. Brad made one lasting contribution to the project of which most people are not aware. It was he who coined the

term "Dendrome", a portmanteau word meaning tree genome. Brad has taken a position at Incyte Corporation in Palo Alto supporting the bioinformatics component of their gene discovery program.

The new curator of the Dendrome Project is Kim Marshall, a name that will be familiar to many. Kim has a BS in forestry from Penn State and an MS in Forest Genetics from the University of Vermont. Kim has worked with me as a research biologist at the Institute of Forest Genetics since 1987. Kim has developed a strong interest in computers over the last few years and when the curator position came open she was a perfect person to follow Brad. I'm looking forward to working with Kim in this new capacity and I know that the users of Dendrome will appreciate the qualities that Kim will bring to the project.

New Publication-New Publication

**Somatic Cell Genetics  
and Molecular Genetics  
of Trees**

edited by  
**M.Raj Ahuja, Wout Boerjan, David B.Neale**

This book covers the latest developments in somatic cell genetics and molecular genetics of trees. The main topics included in this book are regeneration by organogenesis and somatic embryogenesis, gene expression and molecular physiology, molecular markers, genome mapping, gene transfer, and biotic/abiotic stress-related molecular characterizations. The papers in this book have been contributed by experts in the above-mentioned areas.

Contents:

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- IV. Stress-Related Gene Expression

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Dendrome  
Institute of Forest Genetics  
USDA Forest Service  
P.O. Box 245  
Berkeley, CA 94701  
USA