Proceedings

31st Southern Forest Tree Improvement Conference

Sponsored by

The Southern Forest Tree Improvement Committee

Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service, Saucier, MS

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Department of Forestry, College of Forest Resources, Mississippi State University, Mississippi State, MS

June 14-16, 2011

Proceedings of the 31st Southern Forest Tree Improvement Conference

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The papers and abstracts in these proceedings were submitted by the authors as electronic files. Modifications were made in format to provide for consistency and to allow best possible placement of figures and tables. The authors are responsible for the technical content of their respective papers.

Citation for the 31st SFTIC Proceedings:

Nelson, C.D., Rousseau, R.J., and Yuceer, C., editors. 2011. Proceedings of the 31st Southern Forest Tree Improvement Conference; 14-16 June 2011; Biloxi, Mississippi. http://www.sftic.org, 148 p.

Citation of papers in the 31st SFTIC Proceedings:

Authors' Names. 2011. Name of paper. In: Proceedings of the 31st Southern Forest Tree Improvement Conference; 14-16 June 2011; Biloxi, Mississippi. http://www.sftic.org, pp. x-y.

Links to electronic copies may be obtained at: http://www.sftic.org.

Proceedings of the 31st Southern Forest Tree Improvement Conference

Biloxi, MS

June 14-16, 2011

Sponsored Publication No. 53 of the Southern Forest Tree Improvement Committee

Foreword

The 31st Southern Forest Tree Improvement Conference (SFTIC), marking 60 years of biennial technical conferences, was held June 14-16, 2011 in Biloxi, Mississippi under the auspices of the Southern Forest Tree Improvement Committee and in cooperation with USDA Forest Service's Southern Institute of Forest Genetics (Southern Research Station) and Mississippi State University's Department of Forestry. This year's SFTIC provided for three days of stimulating discussions on how advances in genomics and biotechnology, when coupled with traditional breeding practices and outreach/extension activities, can energize forest genetics and tree improvement research, development, and implementation. Just prior to SFTIC, the North American Quantitative Forest Genetics Workshop met and discussed statistical and computational aspects of genomic selection and its opportunities for applications in forest tree improvement. We had 109 colleagues attending SFTIC from seven countries and 37 organizations (14 universities, 17 companies, and six government agencies). Of the 109 attendees, 27 were students (including two undergraduates), and 47 were first time conference participants. A meeting summary is published in Tree Genetics and Genomes (http://dx.doi.org/10.1007/s11295-011-0454-7). We thank all of the presenters and attendees, of which the excellent presentations are documented in these proceedings, the SFTIC Planning and Scientific Committee (listed below), and the financial sponsors (listed below) for making this conference a success. In addition, we thank the folks of Biloxi and the Mississippi Gulf Coast for their wonderful hospitality.

Three awards were presented for outstanding contributions to the conference, and the Southern Forest Tree Improvement Committee thanks these individuals for their contributions:

The **Tony Squillace Award** is given for the best oral presentation based on content, style, and use of visual aids. The winner was John Davis of the University of Florida for his talk entitled "Genome-sequence enabled identification of avirulence genes in the fusiform rust fungus."

The **Bruce Zobel Award** is given for the best oral presentation by a student. The winner was Patricio Munoz of the University of Florida for his talk entitled "The effect of BLUP breeding values in genomic selection accuracy."

The **Belle Baruch Foundation Award** is given for the best poster. The winner was Kevin Potter of North Carolina State University for his poster entitled "Range-wide assessment of genetic structure and variation in eastern hemlock, *Tsuga canadensis*, an imperiled conifer using microsatellite markers." The second and third place poster awards were given to Jake Camp of Mississippi State University and Christine Holtz of the University of Georgia, respectively.

The 31st SFTIC was a success, in part, due to the talent and effort of Dixie Cartwright of Mississippi State University's Division of Academic Outreach & Continuing Education. Our sincere gratitude goes to her.

Finally, we note with sadness the death of one of our pioneering colleagues and leaders, Dr. Bruce Zobel. We dedicate the 31st SFTIC Proceedings to his memory. A brief tribute to Dr. Zobel, kindly provided by Steve McKeand, is included in these proceedings.

The 31st SFTIC Planning & Scientific Committee:

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USDA Forest Service

Randall J. Rousseau (Conference Co-chair)
Mississippi State University

Cetin Yuceer (Conference Co-chair)
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In Memoriam!

Dr. Bruce Zobel, one of the founders of the forest tree improvement community in the southern US, died February 5, 2011 at his home in Raleigh. He was 90.

Dr. Bruce Zobel
1920-2011

Bruce first came to the South as a Marine during World War II where he was stationed in Virginia and North Carolina. At Camp Lejeune near Jacksonville, NC, he was appointed forestry officer. The first professional forestry article he published was "Forestry on a Military Reservation," 1948 Journal of Forestry 46(3):188-190. During his storied career, he Published over 230 articles, authored six books and mentored more than 100 graduate students, many of whom hold leadership positions with universities, government, and industry.

After his military service, Zobel returned to Berkeley to earn masters and doctoral degrees in forestry. His first job in forest genetics came in 1951 when he was hired to run the new program in forest genetics at Texas A&M University.

In 1956, Bruce moved to NC State to lead the newly formed Cooperative Tree Improvement Program. He remained active following his first "retirement" in 1979, founding the Camcore program in gene conservation and forest genetics. He continued to teach undergraduate and graduate classes and mentor graduate students until 2001.

Zobel, who served as the E.F. Conger Distinguished Professor of Forestry, received the O. Max Gardner award in 1972, the UNC Board of Governors' highest faculty honor for outstanding contributions to the welfare of humankind. Recognized as an authority on genetic improvement and variation of wood properties, he earned international recognition in 1975 as the first forester to be awarded the TAPPI Gold Medal for outstanding contributions to the technical progress of the pulp and paper industry.

Bruce was a teacher and mentor without equal. The true mark of excellence in teaching is the ability to teach thinking and independent reasoning; Bruce was unsurpassed in this capacity.

Bruce's accomplishments with the Cooperative Tree Improvement Program in the southern US as well as throughout the world were truly revolutionary. Because of his influence, forestry and the productivity of forest plantations have been forever enhanced. For example, more than 75 percent of the nation's tree planting occurs in the U.S. South. That effort requires planting more than one billion southern pine seedlings each year, of which more than 95 percent of the seedlings are genetically improved. At one time or another, Bruce was directly or indirectly responsible for the majority of that effort.

As tree breeders, we have unique responsibilities. Our legacy to forestry lives in perpetuity. The trees we breed today are the foundation for future forests. Stewardship of our genetic resources is a responsibility that that we cherish and respect. No tree breeder has had a greater influence on forests and forestry than Bruce Zobel. We will miss him, and we thank him.

— Steve McKeand, *June 2011*

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REGIONAL APPROACHES TO SUSTAINABLE BIOENERGY SYSTEMS

William R. Goldner[1] and Jeffrey J. Steiner

[1]Division of Sustainable Bioenergy, Institute of Bioenergy, Climate, and Environment, National Institute of Food and Agriculture (NIFA), USDA, Washington, DC

Region-based production systems are needed to produce the feedstocks that will be turned into the biofuels required to meet Federal mandated targets. Executive and Legislative actions have put into motion significant government responses designed to advance the development and production of domestic biofuels and other biobased products. Thirty-six billion gallons of biofuels must be blended with U.S. transportation fuels by 2022. With more than 12 of the 15 billion gallons of corn grain ethanol presently being produced, careful planning for the expansion of a biomass sector must be done now because the land and financial resources required to produce the next 21 billion gallons of advanced biofuels is significant – an estimated 24 million acres of dedicated feedstock crops and $160 billion to build the needed biorefineries. Increased USDA extramural support brings together robust industry, academic, and government partnerships through the NIFA Agriculture and Food Research Initiative (AFRI) *Sustainable Bioenergy Challenge* Coordinated Agricultural Projects (CAP) program and is coordinated with expanded ARS and Forest Service (FS) intramural research through the regional *USDA Biomass Research Centers* based on directions given by the President's Interagency Working Group report *Growing America's Fuels*. This coordination builds on USDA's research strengths nation-wide to help ensure dependable supplies of feedstocks are available for the production of advanced biofuels to meet legislated goals and market demands using an integrated regional systems approach. Continued research and development for woody biomass crop genetic improvement, as well as innovative sustainable production and logistics are key elements to the potential of a number of sustainable regional biomass systems.

SHAPING THE SOUTH'S BIOFUELS INDUSTRY: ADVANCES IN CONVERSION TECHNOLOGY

Timothy G. Rials[1]

[1]Center for Renewable Carbon, University of Tennessee, Knoxville, TN

The Energy Independence and Security Act of 2007 provided clear direction for reducing the nation's dependence on imported petroleum. With an annual target of 31 billion gallons of renewable fuel, conventional corn ethanol production was capped at 15 billion gallons. The remaining 16 billion gallons is comprised of advanced biofuels. Given the added requirement of greenhouse gas reduction, the roadmap provided clear direction for a major southern contribution to the goal. Because of a perceived technology advantage resulting from a long development history it was largely assumed that cellulosic ethanol would fulfill the demand. Dramatic research and development progress in both the biochemical and thermochemical conversion platforms has altered the landscape in just a few short years. Drop-in fuels that are chemically and functionally similar to today's gasoline are garnering fresh attention. The promise of this new class of biofuels to alleviate the infrastructure incompatibility problems presented by ethanol as a liquid fuel has broadened the range of alternatives that will shape the use of lignocellulosic biomass for renewable fuels and industrial chemicals. Although creating more near term uncertainty on the conversion technology front, this progress has expanded the potential deployment of the industry in the Southeast, where feedstock diversity reigns supreme. This presentation will overview recent developments in biomass conversion technologies, and highlight ongoing barriers to industry expansion.

BIO-OIL'S FUTURE AS A CONSUMER OF MISSISSIPPI TIMBER

Philip H. Steele[1]

[1]Department of Forest Products, Mississippi State University, Mississippi State, MS

Bio-oil, also known as pyrolysis oil, is produced from fast pyrolysis of cellulosic biomass. Fast pyrolysis is a process that heats bio-mass at a moderate temperature (450-550C) and high heating rates in the absence of oxygen for a short time period of less than 2 seconds. The vapors that would normally be combustible are driven off and are rapidly condensed. Water is a major component of the vapors condensed during pyrolysis such that the resulting bio-oil is actually a water emulsion, and not an oil, of chemical compounds composed of the exploded molecular fragments that previously composed the biomass feedstock. The pyrolysis process produces mainly oxygenated compounds and water that result in a high oxygen content that is highly reactive. The oxygenated reactivity of the bio-oil water emulsion causes a number of negative properties, such as high acidity, low energy value, slow ignition and polymerization over time. For these reasons, raw bio-oil must be upgraded by some means to allow utilization as a liquid fuel. The fast pyrolysis process can be performed by a number of reactor types such as Dynamotive's bubbling fluidized bed, Ensyn's moving fluidized bed, KiOR's Fluid Catalytic Cracker (FCC) pyrolytic catalysis process and MSU's auger reactor. Numerous industrial and public research institutions are developing methods of upgrading bio-oil to a drop-in fuel or heating fuel. None of these are commercially operational at this point but some industries are building infrastructure. The properties that geneticists may be developing for producing trees more suitable for cellulosic ethanol production may render the resulting biomass less suitable for production of some bio-oil fuel types. Lignin is an important component in the production of hydrocarbons by the hydroprocessing route for example. High cellulose content produces higher water content in the bio-oil and lower hydrocarbon yields.

WOODY BIOMASS FOR THE PRODUCTION OF ADVANCED BIOFUELS AND BIO-BASED CHEMICALS

Tim Eggeman[1]

[1]ZeaChem Inc., Lakewood, CO.

ZeaChem is a developer of biorefineries for the conversion of renewable biomass into sustainable fuels and chemicals. ZeaChem has partnered with GreenWood Resources, a world leader in development and management of tree plantations, to supply hybrid poplar trees to ZeaChem's demonstration and first commercial biorefineries, located in Boardman, Oregon. Hybrid poplar trees are an excellent biomass source because of their high yield per acre, short rotation and ability to regenerate after harvest, providing superior economic and environmental benefits. ZeaChem's 250,000 gallon per year (GPY) demonstration facility will begin operations in 2011. The first commercial biorefinery is expected to have capacity of 25M GPY and come online in 2014.

INTENSIFYING TREE BREEDING PROGRAMS AND THE IMPACT ON FOREST PRODUCTIVITY AND PROFITABILITY FOR LANDOWNERS IN THE SOUTHERN US

Steve McKeand,[1] J.B. Jett, Saul Garcia, Jim Grissom, Tori Batista-Brooks, Fikret Isik, Josh Steiger, and Ross Whetten

[1]Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC

Maintaining progress in tree improvement has huge economic development implications for the South. Unlike other silvicultural inputs into plantations, the benefits from planting genetically improved seedlings are permanent and spread over millions of acres at minimal extra cost. The economic incentive to increase efforts in tree breeding is impressive. If the genetic gain per year is increased to any extent, the regional financial impacts are worth millions of dollars. For example, the present value (6% interest rate) of a series of continuously improved plantations (1% per year of genetic improvement) was estimated to be $12,255 per planted acre (e.g. a non-ending series of genetically better plantations of one acre being planted each year). If these same plantations were established with the seedlings of only slightly higher genetic quality each year (i.e. genetic gain is increased from 1% per year to 1.1% per year), the present value would be $240 per acre planted per year. For the South where about 1.2 million acres of loblolly pine are planted each year, the increased value to all landowners from this slight increase in genetic improvement would be $300 million.

The NCSU Cooperative Tree Improvement Program continuously works to discover innovative ways to accelerate tree breeding, while reducing costs and increasing efficiency. For example, our group has initiated tandem selection for fusiform rust resistance for elite breeding populations followed by clonal testing to select for growth and quality traits. We are testing new genomic tools for genome-wide estimated breeding values, and we are using markers to construct realized genomic relationship matrices to increase accuracies of breeding values to increase genetic gains. Our fourth cycle breeding strategy, now being developed, will incorporate opportunities to utilize genomic data.

AN EXAMINATION OF THE IMPACT OF IMPROVED GENETIC MATRIAL ON CARBON SEQUESTRATION IN MANAGED LOBLOLLY PINE FORESTS

Mike Cunningham,[1] Phil Dougherty, Chris Maier, and Derek Dougherty

[1]ArborGen, Summerville, SC

Tree improvement's role in increasing productivity and the economic impact of tree improvement on the future value of the Southeastern US wood basket have been described in the *Journal of Forestry* by McKeand et al. 2006. In addition to timber production, managed forests are now being viewed as avenues for increasing carbon sequestration. This presentation will examine how forest genetics is impacting carbon sequestration rates. Five-year results from two studies designed to evaluate how enhanced genetics is impacting production and carbon sequestration will serve as the basis for making projections of genetic effects on rotation length carbon sequestration rates. The potential impacts on increased carbon sequestration that full scale deployment of enhanced genetics could have will be discussed.

PROGRESS AND OPPORTUNUTIES IN VARIETAL FORESTRY

John Pait[1]

CellFor Inc., Atlanta, GA

Loblolly pine varietal forestry has made substantial progress over the last 20 years including maturing field data, development of large scale production systems and demonstrated economic viability. Additionally, low levels of G x E have allowed for robust and stable genotypic performance for growth, disease resistance and log quality traits. Managed forest sustainability and genetic diversity are enhanced with deployment of well-tested varieties in terms of growth and productivity as well as reducing impacts of certain silviculture practices. Tailoring silvicultural systems for some genotypes with crop ideotype crown confirmation and improved growth efficiency can reduce regeneration and management costs. Finally, there are unique opportunities in species conservation and hybridization offered by varietals using the SE platform.

GEONOMIC APPROACHES FOR INCREASING SUSTAINABLE BIOMASS HARVEST IN SOURTHERN FORESTS

Jeffrey F.D. Dean,[1] W. Walter Lorenz, and J. Michael Bordeaux

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

A wealth of genomic sequence information and tools has become available in recent years for loblolly pine (*Pinus taeda*) and an increasingly wider array of other conifer species. These powerful technologies open a wide range of new opportunities to address both short-term and long-term challenges to productivity in southeastern conifer forests. In this presentation we will touch on three case studies in which various genomic tools are being used to address specific problems in loblolly pine. In the first example, spotted DNA microarrays have been used to follow gene expression patterns in different clones of loblolly pine responding to water stress. The work identified networks of genes that responded to water stress in a coordinated fashion, and also identified so-called hub genes that may be master regulators of these network responses. Such hub genes have been recognized as important breeding targets for improvement programs. In the second example, a truncated microarray experiment was used to identify biomarker genes whose expression was induced when loblolly pine was exposed to venom injected into pine trees by the invasive exotic woodwasp, *Sirex noctilio*. Quantitative PCR assays for these biomarker genes were used to demonstrate that Monterey pine (*Pinus radiata*) is about 100-fold more sensitive to the venom than is loblolly pine, and that sensitivity varies by genotype within these two pine species. These findings set the stage for breeding resistance against *S. noctilio* into loblolly pine, which may be important now that *S. noctilio* has become established in North America. Finally, we will revisit an old study of differential gene expression in compression and opposite wood, and use a new database of transcribed gene sequences for loblolly pine to identify genes whose expression is most affected in these different reaction woods. The results highlight genes that contribute to the undesirable qualities of compression wood, and may provide additional targets for advanced breeding efforts.

GENETIC VARIATION IN LOBLOLLY PINE FOR EFFICIENY IN HYDROLYTIC CONVERSION TO ETHANOL

David K. Barker,[1] Steve E. McKeand, Ross W. Whetten, Fikret Isik, and Sunkyu Park

[1]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC

In recent years interest in alternative and renewable fuels has increased considerably. These fuels can help to alleviate environmental issues and serve as replacements for fossil fuels. Ethanol is one alternative fuel already being developed and used that can be made from a variety of feedstocks, including woody plant matter (i.e., lignocellulosic biomass). Woody plant matter can be a good alternative energy source in the Southeastern U.S. due to its wide availability and its ability to grow on marginal sites. As one of the most productive tree species in the Southeastern U.S. as well as being the most planted, loblolly pine (*Pinus taeda* L.) is a logical species of interest for the development of biomass production in the region.

The use of loblolly pine biomass for ethanol production presents some challenges; enzymatic hydrolysis of polysaccharides from softwood pulp typically produces lower yields of fermentable sugars than similar treatment of hardwood pulp. Many chemical and physical wood properties are subject to genetic control, and variation in these properties may well affect the efficiency of ethanol production. In this experiment, 17 clonal varieties of loblolly pine, chosen for a diverse range of chemical and physical wood properties, were tested to characterize variation in yield of fermentable sugars from enzymatic hydrolysis of pulps produced by two different pretreatments. Wood samples from three pooled ramets of each clone were tested, using enzymatic hydrolysis after dilute acid and alkaline pretreatments, to produce data on sugar yields for each clone. Sugar yield is directly correlated with ethanol yield since the sugar is fermented to produce ethanol. The dilute acid pretreatment and enzymatic hydrolysis using 20 filter paper units (FPU) of enzyme produced an average of 210 mg sugar/g wood, with a standard deviation of 20 mg. The alkaline pretreatment and enzymatic hydrolysis using a higher level of enzyme (40 FPU) as well as mechanical beating produced 520 mg sugar/g wood with a standard deviation of 35 mg. A cluster analysis based on near-infrared (NIR) spectra of ground wood samples from multiple ramets of each of the clones was used to divide them into groups. NIR spectra reflect chemical and physical wood properties, so the clustering should have produced groups of clones that are similar for some combination of these traits. The NIR clustering was a significant predictor of sugar yield for the alkaline pretreatment. Given the high heritabilities of most chemical and physical wood properties, these results suggest that it should be possible to identify superior genotypes for biofuel production using NIR analysis.

OPTIMIZATION OF FAST PYROLYSIS PROCESS TOWARDS MORE SUGARS AS AN ALTERNATIVE ROUTE FOR CHEMICALS AND FUELS

El Barbary Hassan,[1] Phillip Steele, Qi Li, and Brian Mitchell

[1]Department of Forest Products, Mississippi State University, Mississippi State, MS

Fast pyrolysis is the most effective and commercially feasible technology for production of bio-oil from biomass. Upgrading of lignin fraction bio-oil into liquid hydrocarbon is the main route for utilization of bio-oil. Increasing the amount of sugars in the aqueous fraction bio-oil will critically increase the importance of this fraction through conversion of sugars into more valuable liquid fuels and chemicals. In this study, a new pretreatment and pyrolysis techniques were applied on green pinewood feedstock. After pyrolysis, both lignin and aqueous fractions bio-oil were fractionated by addition of water. The concentration and average molecular weight of sugars in the aqueous fraction bio-oil were determined by HPLC and GPC, respectively. The yield of sugars in the aqueous fraction bio-oil was increased from 15-24% after the pretreatment. Alfa Laval M20 membrane filtration system was used to separate sugars from the aqueous fraction bio-oil. Factors affecting the separation process such as membrane molecular weight cut off and trans-membrane pressures are still under study.

HIGH THROUGHPUT SCREENING OF CELL WALL COMPOSITION FOR BIOFUELS PRODUCTION

Robert Sykes,[1,2] Kristen Gracom, Geoffery Turner, Evandro Novaes, Gary Peter, Jerry Tuskan, Steve Decker, and Mark Davis

[1]National Renewable Energy Laboratory, Golden, CO [2]BioEnergy Science Center, Oak Ridge, TN

Recent developments in elucidation of lignin biosynthetic pathway have led to the development of new ways of modifying lignin structure, composition, and content. In conjunction with the BioEnergy Science Center funded by DOE, we have developed a surrogate method for estimating Klason lignin content from pyrolysis mass spectra peak intensities. Lignin content is determined from the intensities of selected peaks that have been previously assigned to ions arising from lignin fragmentation and calibrated to known standards. In addition to lignin, a high throughput method was also developed to measure and Glucose and Xylose release after pretreatment using an enzyme assay.

We have used pyrolysis Molecular Beam Mass Spectroscopy (pyMBMS) and in conjunction with sugar release data to analyze large populations for changes in lignin, carbohydrates, and recalcitrance of cell wall material. These two techniques can be combined to rapidly test large numbers of individual clones as well as different feedstocks for biofuel production. In addition, the data obtained from these two methods can be used to identify genes associated with lignin and carbohydrate structure using Quantitative Trait Loci studies.

COLD HARDINESS OF ATLANTIC COASTAL AND PIEDMONT SOURCES OF LOBLOLLY PINE AND THEIR HYBRIDS

Jaime Zapata-Valenzuela,[1] Funda Ogut, Angelia Kegley, W. Patrick Cumbie, Fikret Isik, Bailian Li, and Steven E. McKeand

[1]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC

Planting loblolly pine (*Pinus taeda* L.) outside the natural range or at higher elevations than it normally occurs negatively impacts its survival, growth rate, and wood quality. Cold acclimation in loblolly pine appears to be more influenced by temperature compared to photoperiod. Also, there is interest in examining if inter-provenance hybridization can combine both fast growth and cold hardiness in commercial populations (Alizoti et al. 2006). Seedling growth differences of first-year of Atlantic Coastal and Piedmont provenances of loblolly pine and their hybrids in an outdoor environment in North Carolina were reported by Kegley et al. (2004). These results were verified at four years, when height and volume were assessed in five Piedmont regions (Alizoti et al. 2006). However, it was not confirmed that the hybrid populations would exhibit acceptable cold hardiness. The objective of this study was to evaluate differences in cold hardiness between one year-old seedlings of Atlantic Coastal sources, Piedmont sources, and their hybrids, after artificial freezing.

Materials and Methods

Seedlings of 59 polycross families representing two within provenance hybrids (Coastal x Coastal or CxC, Piedmont x Piedmont or PxP), and two inter-provenance hybrids (Coastal x Piedmont or CxP, Piedmont x Coastal or PxC) were used in this study. The preconditioning treatments consisted of three acclimation regimes designed to reflect climatic conditions in three deployment regions for loblolly pine. After acclimation, the seedlings were subjected to a standard freezing treatment. The plants were moved to a Delfield-Alco® 6000 series freezer to expose them to a controlled drop in temperature. Temperature was reduced at a rate of 3-5ºC per hour until the target -15ºC was reached. After thawing, the plants were removed to an outdoor facility for symptoms to develop. Initial height (cm) was measured before the freezing treatment as an indicator of plant vigor. Rating of injury occurred 10-14 days after freezing exposure, with one person evaluating all trees. Freezing injury I (%) of the entire seedling was measured as percent foliage dead. Final survival and vigor (%) of the trees was assessed in the spring when the plants began flushing. Elongation of the actively growing portion of the stem was measured as flush length (cm). Mortality percent of the trees and a secondary injury II (%) evaluation were assessed. Variance components for each trait were obtained by REML procedure using ASReml (Gilmour et al. 2009). Individual narrow-sense heritabilities pooled across populations and genetic correlations between pairs of traits were estimated as the ratio of the additive genetic variance and total phenotypic variance for each trait. We also examined relationships among response variables using family mean correlations obtained from Pearson product-moment correlations.

Results and Discussion

For injury and growth traits, acclimation treatment effects were not significant, and there were no significant interaction with population. The implication is that a single preconditioning treatment could be applied to different families regardless of origin. The relatively short time of the acclimation and no difference in photoperiod may have also contributed to the lack of treatment effect. In this experiment, differences in cold hardiness were important among and within populations. The PxP seedlings had increased hardiness and survival relative to the other populations. Most families with high injury had a Coastal maternal parent, whereas most families with the least injury had a Piedmont maternal parent. For the hybrids, it appeared that maternal influence was greater than the paternal influence for cold-hardiness, and the PxC population was as cold hardy as PxP. Differences in injury between the populations probably resulted from differences in timing of initiation of the acclimation process; PxC and PxP may have begun acclimating sooner than the CxC as part of their adaptive strategy. Kegley et al. (2004) and Alizoti et al. (2006) used the same families to determine populations effects on growth traits, and differences between pure CxC and PxP material were found: family differences in one year-old seedlings were observed, and some fast-growing PxP families could be used in controlled crosses for hybrid selection. The CxC and CxP populations exceeded growth of both PxC and PxP families at four years growing in different regions, although their advantage decreased as the regions became more inland or with harsher environmental conditions. The PxP material survived significantly better than coastal source provenances when planted in cold area regions, probably because of natural cold adaptation.

Narrow-sense heritability pooled across all populations was higher for injury II than injury I. Estimates were variable for each population, ranging from 0.02 to 0.43 for injury I and II. In general the CxC families exhibited higher heritability for the traits measured, followed by the PxC for injury I and the PxP hybrid for injury II. Previous experimental results suggest that cold damage traits are under moderate genetic control. We found higher family mean heritability for initial height in the PxC and PxP material, whose estimates had small standard errors. These results suggested that families from Piedmont parents could be selected in a breeding program for both growth and cold tolerance more efficiently than families from the CxC source. Kegley et al. (2004) found similar trends at first year height in outdoor conditions, although their plants were not exposed to cold.

The strong negative genetic correlation between flush length and injury II (-0.89) can be considered favorable: trees with less freeze damage had longer flushes. The Figure 1 showed that this trend was consistent for family means within populations with an overall phenotypic correlation of -0.74; while CxC and CxP families seemed to be scattered ranging from 40% to 100% injury, PxC and PxP families clustered within 30% to 60% of damage with few exceptions. Also, the growth recovery of elongated stem tissue was faster with reduced damage, which was also reflected in the high flush length mean for PxC and PxP populations. Howe et al. (2003) summarized several literature results where correlation between cold damage and flush increment is more consistent across populations, but weaker and more variable within populations. Our results within populations were more consistent than observed in previous studies, which might be important to detect stable families for advanced selection strategies.

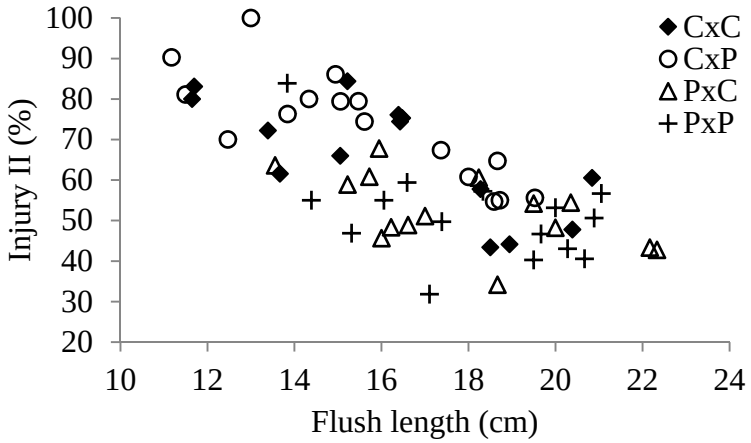

Figure 1. Scatterplot of family means between injury II and flush length for the Atlantic Coastal (CxC), Coastal x Piedmont (CxP), Piedmont x Coastal (PxC) and Piedmont (PxP) populations. The family mean correlation across all the populations was -0.74.

In summary, this freezing experiment showed that inter-provenance hybrids of loblolly pine have potential as planting stock for Piedmont sites. The CxP hybrids were cold injured, but they could grow as well as pure CxC families. On the other hand, PxC hybrids were as cold hardy as the pure PxP families. Also, there were large differences among families within populations for cold hardiness traits. The large variation suggested that cold hardiness could be improved by family and within population selection in a breeding program to combine better growth of Coastal sources with the superior cold tolerance of Piedmont sources into hybrids populations. However, selection impact seems difficult to predict due to variability within populations and probably because of genetic control of multiple genes with small effects. Additional studies are required to identify cold tolerant hybrid families that would allow for both cold hardiness and superior growth to be improved through breeding and selection.

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THE RATE OF HYBRIDIZATION AND INTROGRESSION BETWEEN LOBLOLLY PINE (*PINUS TAEDA* L.) AND SHORTLEAF PINE (*PINUS ECHINATA* MILL.) HAS INCREASED MARKEDLY SINCE THE 1950S.

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Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) are important forest species that have large ranges across the southeastern United States that share a large sympatric range in addition to their own allopatric ranges. The two species have been crossed artificially (Schreiner 1937), and natural hybrids have been observed (Hare and Switzer 1969). It is thought that hybridization is normally prevented by the different flowering time in the two species, but when the climatic conditions are right, hybridization may occur. Earlier studies used morphology and later isoenzymes to identify natural hybrids, but recent studies have used DNA markers to identify hybrids. Xu et al. (2008a, b) reported hybrids in study samples from material grown from seed collected in the 1950s from the Southwide Southern Pine Seed Source Study (SSPSSS) using amplified fragment length polymorphism (AFLP) markers. Stewart et al. (2010) followed up on that study using short sequence repeat (SSR) markers, also called microsatellite markers, to identify hybrids in the same source material.

In this study, we used microsatellites to characterize the hybrid status of trees collected from current stands from the same counties that were represented in Xu et al. (2008a, b) and Stewart et al. (2010). The goal of this study is to compare the rates of hybridization and introgression in modern stands to those from the 1950s. From the 1950s to present, the rates of hybridization and introgression in both species have increased dramatically. Introgression can be a major threat to species, even leading to extinction, and increased introgression in many species has been connected to human activities (Wolf et al. 2001).

Materials and Methods

Green leaves from both species were collected by foresters in the same counties as those collected for the studies by Xu et al. (2008a), Xu et al. (2008b), and Stewart et al. (2010) i.e., the SSPSSS. Loblolly pine samples were collected from 9 counties east of the Mississippi River and 2 counties west of the river, and shortleaf pine samples were collected from 6 counties east of the Mississippi River and 4 counties west of the river. DNA was extracted from the needles using the Qiagen DNEasy Plant Minikit (Qiagen, Valencia, CA.)

Twenty-five microsatellite markers previously confirmed to be polymorphic in both speices were used in this study. Three primers for these markers were used during PCR, two that flanked the short sequence repeat region and one primer labeled with a fluorophore. All PCR products were scored using a LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE).

Structure version 2.3.2 was used to determine hybrid character of individuals. We set population number k to 2, which represents the two species analyzed in this study. Hybrids were reported when predicted genome proportion levels (*Q*) were between 0.9531 and 0.0469, about what is expected for trees in an F1 cross or a first through third backcross generations.

Results and Discussion

The rates of hybridization and introgression increased markedly in both species: 27.3% hybrids in loblolly pine populations and 45.7% hybrids in shortleaf pine populations compared to rates of 4.5% and 3.3%, respectively, in the 1950s populations. West of the Mississippi River, the shortleaf pine hybridization rate increased from 7.5% to 54.0%, and the loblolly pine hybridization rate increased from 9.1% to 20.0%. East of the Mississippi River, the hybridization rate for shortleaf pine increased from 0% to 40.0%, and the hybridization rate of loblolly pine increased from 2.2% to 29.2%.

Introgression is a known cause of extinction of species—or, to be more precise, genomes (Allendorf et al. 2001). In general, hybridization can threaten a taxon in a wide variety of ways, through the generation of poorly adapted hybrids, the generation of hybrids with greater vigor than one or more of the contributing species, or the introgressive extinction of one or more species (Simberloff 1996). Discovering whether introgression is a natural process or anthropogenic is crucial to understanding how or whether to manage the issue (Allendorf 2001). Given the timescale for change in introgression in this study (about 50 years), it is almost certain that the cause is, at least in large part, human caused in this case.

Human causes for introgression include introduction of plants and animals, habitat fragmentation, and habitat modification (Allendorf & Luikart 2007). All three could have an impact on loblolly pine, shortleaf pine, and their hybrids. Loblolly pine is being planted outside of its range, as well as being planted as a replacement for lost/harvested shortleaf pine stands, and there is evidence that shortleaf pine genes have been introgressing into the allopatric loblolly pine populations. Habitat fragmentation is common in the southeastern United States, a factor that can lead to the mixing of previously distinct gene pools (Rhymer & Simberloff 1996). In the case of loblolly pine and shortleaf pine, habitat fragmentation could lead to more opportunities for cross-pollination. As both species are early successional pines, they will often invade the disturbed sites generated by human development, a process that can create a corridor for the two species to more often enter each other's habitat (Rhymer & Simberloff 1996). These corridors may change the frequency of contact and encourage introgression by becoming hybrid zones, or regions where two species often intercross to create hybrids (Wolf et al. 2001). One other important form of habitat modification for this case is the planting of loblolly pines in shortleaf pine habitats, often as replacement trees for lost/harvested shortleaf pine stands.

The ecology of loblolly pine and shortleaf pine is rapidly changing, as human activity and forest management make their marks on the distribution of these two species. It appears that hybridization and introgression are phenomena with increasing effects on both pine species, and the future of these two species is difficult to ascertain. Through habitat modification, global warming, fire suppression, seed/seedling movement through artificial regeneration, mankind is

altering the genetic makeup of loblolly pine and shortleaf pine. While it is beyond the scope of this study, management practices regarding these two species need to be reexamined to determine their ecological efficacy.

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RESULTS FROM A QUANTITATIVE GENETICS STUDY OF PHYSICAL WOOD PROPERTIES OF *PINUS PATULA*

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Tree improvement programmes for forestry species started in Southern Africa during the 1950s. In the first two generations of breeding, volume improvements of between 10 and 30% have been achieved, and the future focus of many programmes has moved to the improvement of wood properties. This study utilized half-rotation age *Pinus patula* material grown in Zimbabwe by the Zimbabwe Forest Commission from a full diallel mating design and additional factorial crosses. The sampled progeny trials were planted on several sites with a range of altitudes. This presentation provides some background to this wood and fibre properties project and present results from the diallel mating design. Physical wood properties such as pith-to-bark wood density, cell anatomy and fibre properties were studied and their genetic control was quantified. Wood density was determined using x-ray densitometry and cell anatomy was studied with image analysis, while fibre properties were determined using the MorFi© fibre analyzer system. Genetic parameters for density, cell anatomy and fibre properties are presented. This study forms part of a bigger project which aims to identify the physical wood and fibre properties under genetic control, and identifying an early screening method to include selection for these properties at half-rotation age.

ASSOCIATION MAPPING OF ADAPTIVE AND BREEDING TRAITS IN EAST TEXAS LOBLOLLY PINE (*PINUS TAEDA* L.) BREEDING POPULATIONS USING HIGHT-DENSITY SNP GENOTYPING

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Loblolly pine (*Pinus taeda* L.) is the most commercially and ecologically important tree in the Southeastern US and is the main species in the Western Gulf Forest Tree Improvement Program (WGFTIP), one of the largest tree improvement programs in the US. Recent availability of genomic markers through the Conifer Translational Genomics Network (CTGN) has enabled a genome wide survey of population parameters in the WGFTIP loblolly pine breeding populations reported here.

Materials and Methods

The study included first- and second-generation selections from the WGFTIP East Texas breeding population. The first-generation selections were from natural stands and plantations originating at the western limit of the natural distribution of loblolly pine. The first-generation selections were subsequently partitioned into sublines and subjected to breeding and controlled pollination. Their progeny contributed the second-generation selections. Genome wide variation, population substructure and adaptive trait associations were investigated in both first- and second-generation populations using single nucleotide polymorphism (SNP) markers developed through the CTGN.

Genetic variation and its partitioning within the breeding populations were analyzed in 1,706 trees using 4,264 SNPs. These SNPs are based on amplicons representing partial sequences of ~3,000 expressed genes and were originally discovered in a small range-wide population set in the NSF funded ADEPT2 resequencing project. The tree samples were subdivided into 14 (first-generation) and 8 (second-generation) populations based on their geographical origin and 30 breeding groups based on their pedigree and the WGFTIP breeding strategy. Population structure was analyzed using Bayesian analysis as implemented in software *STRUCTURE* (Pritchard et al. 2000) and the ΔK parameter of Evanno et al. (2005). Individual computer runs for each putative cluster were permuted using LargeKGreedy algorithm implemented in the *CLUMPP* software (Jakobsson & Rosenberg 2007) and then visualized using the *DISTRUCT* (Rosenberg 2004). F_{ST} outlier method was used to detect candidate markers with alleles that were putatively affected by natural selection. The blast homology search was done to assess their functional significance. Significant associations between markers and adaptive traits were also studied using *TASSEL* (Bradbury et al. 2007). Haplotypes were reconstructed using the *fastPHASE* program (Scheet & Stephens 2006), and linkage disequilibrium (LD) between SNPs in all 12 linkage groups was estimated using HAPLOVIEW program (Barrett et al. 2005).

Results and Discussion

Population Substructure

Population structure appears to be weak as indicated by *STRUCTURE* analysis. The log probability of data suggests the number of clusters to be between 3 and 6, and ΔK suggests the number of clusters to be no more than 6 (Figure 1). Given the uniformity of the environment across the region and the limited area sampled, population structure appears to be mostly subtle if not an artifact of sampling.

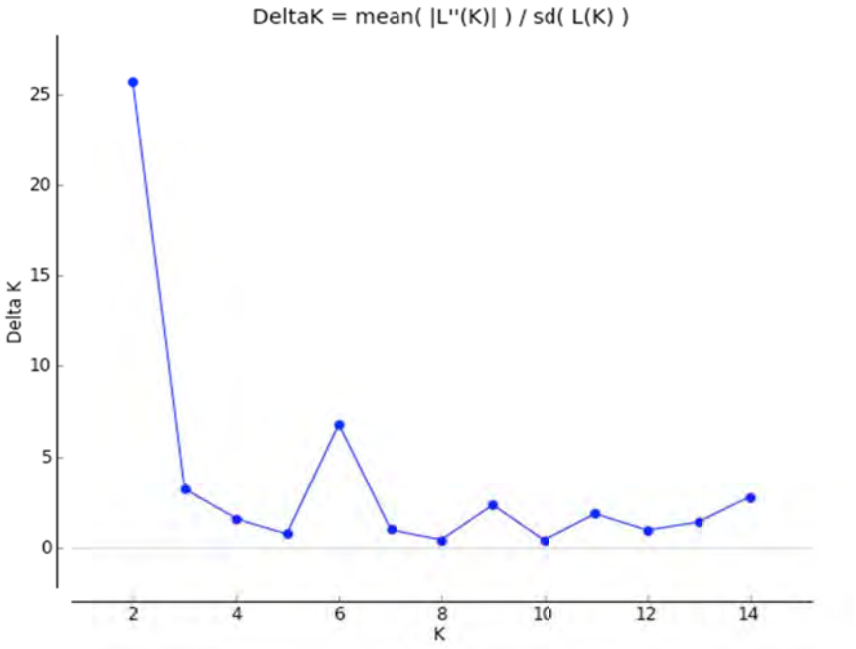

Figure 1. ΔK Estimator of population structure (Evanno et al. 2005).

Signatures of Natural Selection

F_{ST} outlier analysis for all 4,264 marker genotypes in the first generation samples revealed several markers that contributed to extremely high or extremely low F_{ST} estimates. Allelic variation in these markers demonstrates signatures of possible balancing or diversifying selection. Detailed functional annotation has been done for these markers.

Genome-Wide Linkage Disequilibrium

With approximately 100 SNPs per linkage group mapped using a relatively small segregating population (Eckert et al. 2009), the map resolution was insufficient to observe the rate of LD decay (Fig. 2). Most LDs were observed between closely linked SNPs, but there were a few significant LDs observed between markers separated by considerable distances. We hypothesize

that this could be due to several reasons including unaccounted family structure, population substructure, mapping errors and epistasis.

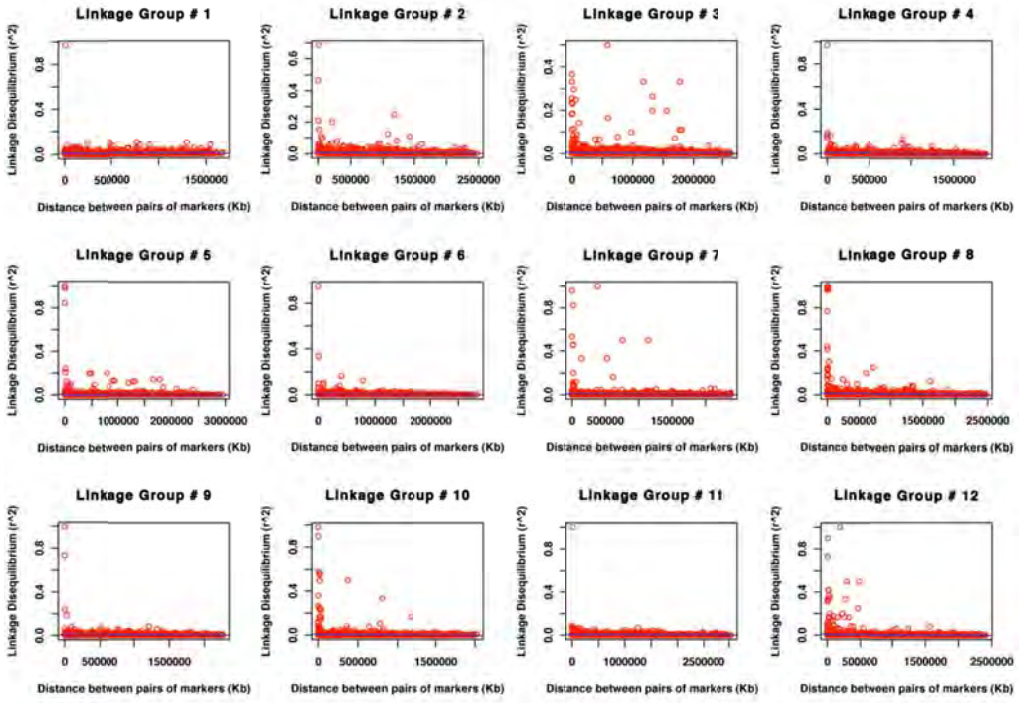

Figure 2. Pairwise LDs for SNPs mapped in 12 loblolly pine linkage groups.

Association Mapping Analysis

Significant associations of SNPs with several phenotypic traits such as height, diameter, survival on droughty sites, stem forking, wood specific gravity, etc. were detected. Sequences with SNPs that resulted in such associations were further annotated using their homology with functional genes in related and model species. Examples of associated genes based on BlastX functional annotation include decarboxylases, reductases, RNA polymerases, stress proteins, beta tubulins, chlorophyll binding proteins, metallothionein-like proteins, CDC2 protein kinases, arabinofuranosidases, Acyl CoA synthetase, sodium symporter, serine-rich proteins, phosphoglyceride transport proteins etc.

Conclusions

The SNP diversity is relatively high in the studied populations. Inbreeding is low, and many populations have excess of heterozygotes, especially in second-generation selection populations. Population differentiation is low in natural stands but higher among second- generation populations and breeding groups, attributable to their relatedness imposed due to the breeding strategy. Population substructure is relatively weak, but there could be up to 6 subpopulations. SNPs contributing to extremely high or low F_{ST} were detected and may exhibit signatures of selection. Numerous associations were detected between SNPs and adaptive phenotypic traits,

but most of them failed the false positive rate test. There are no long-distance LD blocks in the current population, but current SNP density is insufficient for tracing the rate of LD decay. The relatively sparse SNP resolution suggests insufficient power for detecting most associations between current SNP markers and QTLs.

Acknowledgements

The authors acknowledge the support from Texas A&M University Genetics graduate program, the USDA, the NSF, the Texas Forest Service & Western Gulf Forest Tree Improvement Program and the SFTIC early career travel grant to Vikram Chhatre.

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GENETIC VARIATION IN PINES INFLUENCING ECTOMYCORRHIZAL SYMBIOSIS: POTENTIAL IMPLICATIONS FOR GENOTYPE SELECTION AND SOIL CARBON SEQUESTRATION

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Ectomycorrhizal (ECM) fungi provide one of the main pathways for carbon (C) from pines into soils, where these fungi make significant contributions to microbial biomass and soil respiration. However, ECM fungal species vary significantly in traits that likely influence C sequestration, such that forest C sequestration potential may be driven in part by the community composition of ECM fungi. In three recent experiments we found evidence for genetic variation in *Pinus* species controlling ECM fungal community composition, suggesting the potential to influence ECM community composition through pine genotype selection. A bishop pine (*P. muricata*) population in California was shown to harbor significant genetic variation for compatibility with one common ECM fungal species exhibiting a high-biomass exploration strategy, *Rhizopogon occidentalis*. Native populations of Monterey pine (*P. radiata*) were shown to exhibit significant differences in compatibility with three different ECM fungal species in the family Pyronemataceae. A loblolly pine (*P. taeda*) common garden pedigree population exhibited substantial narrow-sense heritability for compatibility with several ECM fungal species, and negative genetic correlations among fungal species differing in exploration biomass. Altogether, these results suggest that selection of particular *Pinus* genotypes could alter the community composition of symbiotic ECM fungi in managed southern pine forests, potentially influencing soil C sequestration.

EFFECT OF INBREEDING IN ELITE FAMILIES OF LOBLOLLY PINE

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Inbreeding can increase rapidly in intensively selected breeding populations and is detrimental in outcrossing species such as loblolly pine (*Pinus taeda* L.). Managing inbreeding and the deleterious effects on metric traits is a primary objective in forest tree breeding programs. Effects of inbreeding were quantified for 10 Coastal and 10 Piedmont loblolly pine families. Each of 10 selected parents was bred to other related and non-related selections to provide a gradient of inbreeding coefficients (F); F = 0, 0.125, 0.25, 0.5. Progeny from each cross were planted in field trials, and periodic measurements were collected. There was generally a linear decrease in metric traits with increase in inbreeding coefficient. However, there was a significant family by inbreeding coefficient interaction; not all families responded the same to inbreeding. Further analyses are underway to identify, describe, and explain the nature of differences among families to inbreeding.

ASSOCIATION GENETICS AND MARKER EFFECTS FOR GROWTH AND STEM QUALITY IN LOBLOLLY PINE

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An association genetics approach was taken to identify single nucleotide polymorphisms (SNPs) associated with variation in growth and stem form traits in loblolly pine (*Pinus taeda* L.). Associations were tested between 4,200 SNPs and breeding values in a population of 200 largely unrelated selections of loblolly pine. We report SNP-phenotype associations for sawtimber, volume, and stem straightness. Significant SNPs will be used to estimate genetic values for an independent population of clonally replicated trees. We will report correlations between marker-based and phenotypic-based genetic values and potential applications.

INTROGRESSION OF LOBLOLLY PINE ALLELES INTO SLASH PINE; QTL ANALYSIS FOR CROWN, GROWTH, AND GROWTH EFFICIENCY IN A PSEUDO-BACKCROSS ((SLASH X LOBLOLLY) X SLASH) FAMILY

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Although slash and loblolly pine are closely related species, they have distinct differences in site preference, crown architecture and stem growth. The objectives of this research are to determine the effect of introgressed loblolly pine alleles into slash pine and to identify quantitative trait loci (QTLs) from the loblolly pine donor to select the best individuals for future introgression into slash pine breeding populations. A single field test was planted with one pseudo-backcross between an F1 hybrid of (*Pinus elliottii* x *Pinus taeda)* x *Pinus elliottii* (BC1) and families of the species progenitors.

Phenotypic analyses of third-year size showed differences in growth efficiency in the BC1 compared with the pure species. The different growth strategies of the two pure species gave BC1 progeny an advantage in crown architecture, yielding greater stem volume per unit crown size with fewer primary and secondary branches and less taper than loblolly pine.

The BC1 population, 490 individuals, was genotyped for 4300 single nucleotide polymorphisms identified in loblolly pine. Eight hundred and two informative markers were used to construct a map containing the 12 linkage groups. QTLs were discovered for growth, crown architecture, survival and growth efficiency, which explain a significant proportion of the variation.

BIOTECHNOLOGY OF REPRODUCTIVE ONSET: A NEW ERA FOR ACCELERATED TREE BREEDING?

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When compared with many annual crop plants, tree domestication is in its infancy. One of the main reasons for the slow progress towards tree domestication is that the lengthy juvenile period prevents trees from early sexual reproduction that is needed to develop pedigreed offspring. Thus, the control of reproductive onset is of great scientific and commercial importance. Recent functional genomics studies have begun providing a framework for how first time and seasonal reproduction are regulated in poplar (*Populus* spp.). In particular, *FT* and *TFL* genes and their associated networks in signaling pathways are providing great insights into reproductive onset and seasonal cycles of reproduction. Our objective in this presentation is to update the community on how genetic, physiological, and environmental factors collectively regulate the onset of reproduction in poplar. The discussion will include how this knowledge can be used by breeders and biotechnologists to speed breeding to improve tree growth and development.

PHYSICAL LOCATION OF 18S-28S AND 5S RIBOSOMAL RNA GENES IN CHINESE CHESTNUT (*CASTANEA MOLLISSIMA*)

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For the current analysis we report on the distribution of the ribosomal rRNA genes (i.e., rDNA loci) in Chinese chestnut. Two sites of 18S-28S rDNA (one major and the other minor) and one site of 5S rDNA were identified, and each is located on a different pair of homologous chromosomes. Earlier we reported similar results for these rDNA loci in American chestnut. However, we note here that the chromosome pair with the major 18S-28S rDNA locus contains a much larger satellite (i.e., chromosome region between major 18S-28S locus and the telomere) in Chinese chestnut than in American chestnut.

Introduction

The American chestnut (*Castanea dentata,* $2n=2x=24$), once known as the "King of the Appalachian Forest", was nearly destroyed by *Cryphonectria parasitica* (causing chestnut blight disease) a fungal pathogen accidentally introduced from Asia in the late 1800s (Hepting, 1974). In contrast to American chestnut, Chinese chestnut (*C. mollissima,* $2n=2x=24$) is resistant to *C. parasitica*. Utilizing American chestnut trees (mostly stump sprouts) still found in the wild, The American Chestnut Foundation (TACF) is transferring resistance from Chinese chestnut into American chestnut through backcross breeding. To facilitate this work our lab has teamed up with TACF to carryout cytogenetics research. We have been successful in preparing chestnut chromosome spreads from somatic tissue (root-tip meristems) and reproductive tissue (pollen mother cells) and applying fluorescent *in situ* hybridization (FISH) techniques to localize genetic elements on particular chromosomes. FISH analysis along with traditional cytogenetics is revealing important details of the structural organization of the chestnut genomes. Ribosomal RNA gene families (18S-28S rDNA and 5S rDNA) provide valuable cytological landmarks for karyotyping and studying the relationships between species and genera (Heslop-Harrison et al., 1992). Earlier we reported that American chestnut has two 18S-28S rDNA sites, one major and the other is minor, and each is located on a different pair of homologous chromosomes (Islam-Faridi et al., 2009). We also reported that the 5S rRNA gene is located interstitially on a third chromosome pair. Here we provide a preliminary report on the physical location of the rRNA genes in Chinese chestnut.

Materials and Methods

Germinating seedlings of the Chinese chestnut cultivar 'Veselicky' were grown in MetroMix potting soils (SunGrow SB-650) in a greenhouse in College Station, TX. Actively growing root tips, about 1 cm long and appearing milky white and transparent, were harvested into either 2.5 mM hydroxyquinoline solution or 0.8% α-bromonaphthalene aqueous solution, pre-treated for 2

h in the dark to accumulate metaphases, and then fixed in 4:1 95% EtOH:GAA. The root-tips were then enzymatically processed to prepare chromosome spreads as described elsewhere (Jewell and Islam-Faridi 1994), except that the enzyme solution was modified to the following: 40% (v/v) Cellulase (C2730, Sigma), 20% (v/v) Pectinase (P2611, Sigma), 40% (v/v) 0.01 M Citrate buffer, 2% (w/v) Cellulase RS (SERVA Electrophoresis GmbH), 3% Cellulase R10 (Yakult Pharmaceutical, Japan), 1% Macerozyme (Yakult Pharmaceutical, Japan) and 1.5% Pectolyase Y23 (Kyowa Chemical, Japan).

Whole plasmid DNA with 18S-28S rDNA insert of maize or 5S rDNA insert of sugar beet including the spacer region were labeled with biotin-16-dUTP (Biotin-Nick Translation Mix, Roche, Germany) or digoxigenin-11-dUTP (Dig-Nick-Translation Mix, Roche, Germany) following the manufacturer's instructions. Agarose gel electrophoresis was used to check the fragment sizes of the probe DNA and labeled nucleotide incorporation was verified by dot-blotting.

Standard FISH technique was utilized as previously reported (Hanson et al., 1996; Islam-Faridi et al., 2002). Sites of biotin-labeled probe hybridization were detected using Cy3-conjugated streptavidin (Jackson Immuno Research Laboratories, USA). Sites of digoxigenin-labeled probe binding were visualized using fluorescein-conjugated sheep anti-digoxigenin (Roche, Germany). Slides were counter stained with DAPI (4 ug/ml) in McIlvaine buffer (pH 7.0) including a small drop (10 ul) of Vectashield (Vector Laboratories, USA) to prevent photo-bleaching the fluorochromes.

Chromosome spreads were viewed under a 63X plan apo-chromatic objective and digital images were recorded using an epi-fluorescence microscope (AxioImager M2, Carl Zeiss, Germany) with suitable filter sets (Chroma Technology, USA) and a CoolCube 1 high performance CCD camera. Images were pre-processed with Ikaros and ISIS v5.1 (MetaSystems Inc., USA) and then further processed with Adobe Photoshop CS v8 (Adobe Systems, USA).

Results and Discussion

Our data show that we were successful at preparing chestnut somatic chromosome spreads from root-tips meristems on ethanol-cleaned glass slides. The chromosomes are sharp and distinct and all are observed to be in unifocal position (e.g., see Fig. 1). All 24 chromosomes of Chinese chestnut can easily be separated from each other with essentially no overlap. We occasionally see 26 chromosomes, mainly from prophase to early metaphase. The extra two apparent chromosomes are actually detached satellite regions (i.e., region distal of the major 18S-28S rDNA locus) from the homologous pair of satellited chromosomes. FISH with 18S-28S rDNA probe showed that the satellite region is connected with the nucleolus organizer region (NOR), the site of the rRNA gene, as revealed by the 18S-28S rDNA signal (Fig. 1).

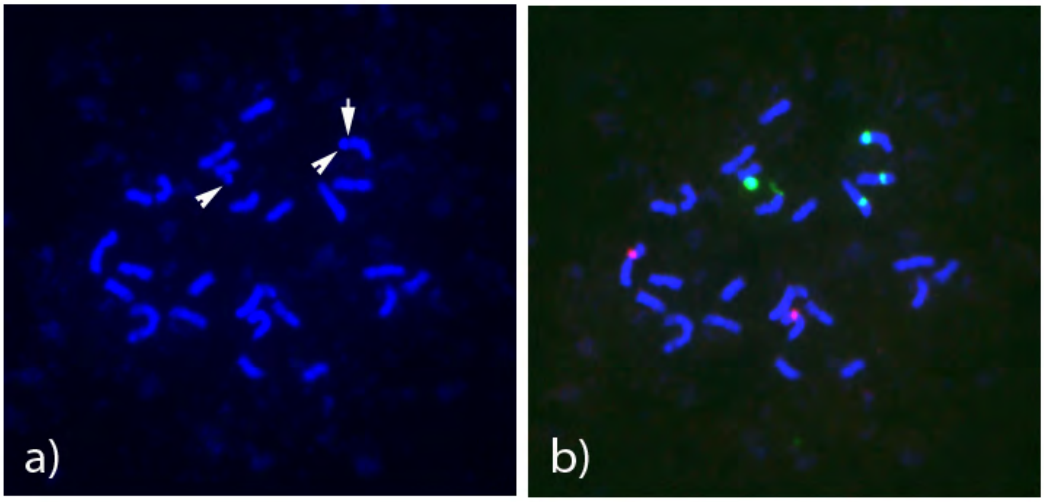

Figure 1. Images of FISH using rDNA probes (18S-28S and 5S) on somatic chromosome spread of Chinese chestnut cultivar 'Veselicky': (a) DAPI image of metaphase chromosomes, arrow shows the NOR (the site of 18S-28S rDNA, green signal in 1b) and arrowheads show the satellite regions; (b) the same chromosomes as in 1a with 18S-28S and 5S rDNA FISH signals (green and red, respectively).

We have observed two sites of 18S-28S rDNA (one major and one minor) and one site of 5S rDNA in Chinese chestnut cultivar 'Veselicky' (Fig. 1b). Similar results were also reported in American chestnut (Islam-Faridi et al., 2009). The satellite region in Chinese chestnut is clearly larger than its counterpart in American chestnut. The larger satellite region in Chinese chestnut might have resulted from an unequal cross-over. In addition, our results suggest that there might be a second 5S rDNA locus in Chinese chestnut. Additional research involving other Chinese chestnut cultivars is being carried out to confirm the second 5S rDNA locus and to evaluate size of the satellited region and other possible variations in the major 18S-28S rDNA locus.

Based on the above results, we conclude that these two species are structurally different from each other with respect to their satellited chromosome. This structural difference can serve as a marker indicating species origin of this chromosome in any individual tree, which can be especially useful in interspecies hybrid backcross breeding programs. It remains to be determined whether species heterozygosity at this rDNA locus will be beneficial or not, or if any important QTLs or genes are linked to the locus.

Acknowledgements

We thank Fred Hebard and Paul Sisco for providing seeds of various Chinese chestnut cultivars used in this research.

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DELIVERY AND CHARACTERIZATION OF CANDIDATE GENES FOR DISEASE RESISTANCE IN AMERICAN CHESTNUT: VECTOR CONSTRUCTION AND SCREENING

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American chestnut (*Castanea dentata*) was an abundant tree and a keystone species of eastern US forest ecosystems. "It has been said that an enterprising squirrel could once travel from Maine to Georgia on the interlocking branches of chestnut trees" (Anon). A blight caused by the fungal pathogen *Cryphonectria parasitica* was introduced to the eastern US around the turn of the 20th century. The pathogen spread rapidly and, although root sprouts persist, the American chestnut was essentially lost from the eastern forests by the mid-1900s. The near extirpation of American chestnut has been considered one of the worst ecological disasters in North American history.

Restoration of American chestnut has been selected as the Forest Health Initiative's first project to explore the potential of biotechnology for addressing threats to forest health (Nelson et al. 2009). The multi-institutional collaboration is focused on development and application of biotechnologies for production of disease resistant American chestnut germplasm. Chinese chestnut (*Castanea mollissima*) is resistant to chestnut blight and candidate genes for resistance to pathogens such as *Cryphonectria* and *Phytophthora* have been identified in Chinese chestnut through genomic and bioinformatic approaches. A number of genes conferring anti-fungal activity have been reported in other plant species as well.

Current approaches to obtain candidate resistance genes fall into three broad categories. The first has been comparative transcriptomics of *Cryphonectria*-inoculated American and Chinese chestnut. High-throughput sequence analyses of genes expressed in the two chestnut species have identified specific gene transcripts that are differentially regulated in Chinese chestnut relative to American chestnut. Copies of identified genes were isolated from Chinese chestnut cDNA libraries. The second approach, which also targets genes from Chinese chestnut, is to map quantitative trait loci (QTL) conferring resistance and integrate these data with high-throughput genomic sequencing to identify candidate resistance genes located within the QTL intervals. The third approach is to leverage advances from fungal pathogen resistance studies in other plant species to identify heterologous candidate resistance genes for transformation and testing in American chestnut.

To evaluate the relatively large number of candidate genes that have been identified for *Cryphonectria* and *Phytophthora* resistance, an efficient system for cloning, transformation, expression, and molecular characterization was developed. Variation in delivery and expression of individual genes is minimized through standardization of genetic elements and inter-element sequences so that phenotypic characteristics conferred by individual candidate genes can be

evaluated. A modular vector design facilitates gene insertion, multi-gene construct assembly, and the potential for integrating genome-derived promoter-gene regions.

A binary vector, *pFHI-03*, was constructed for use as a standard, constitutive expression vector for *Agrobacterium*-mediated transformation of chestnut embryogenic cultures (Figure 1). The vector includes the *npt II* gene driven by the Ubiquitin-10 promoter from *Arabidopsis* for antibiotic selection of transformed chestnut cultures. The Ubiquitin-11 promoter from *Arabidopsis* is included to drive expression of the candidate gene of interest. A multiple cloning site is integrated at the 3' end of the promoter and is followed by the CaMV 35S terminator sequence. The *Pac I* recognition sequence within the multiple cloning site provides an invariant AT-rich sequence fusing the 3' end of the promoter directly to the methionine initiator codon sequence of the inserted candidate gene. Unique restriction sites border the elements of the vector to facilitate substitution of promoters, terminators, and selectable markers. These unique restriction sites also facilitate modification of the vector for multi-gene delivery and expression.

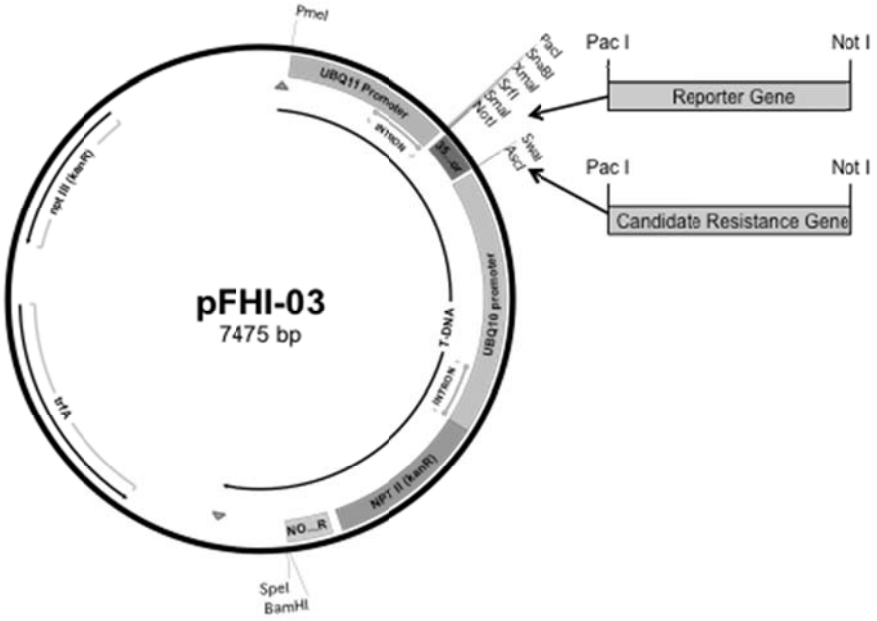

Figure 1. Map of the *pFH-03* vector. A multiple cloning site bordered by *Pac I* and *Not I* recognition sequences is located between the 3' end of Ubiquitin-11 constitutive promoter and the 5' end of the CaMV 35S terminator. A candidate gene or a reporter gene was inserted at the *Pac I* and *Not I* sites to produce individual vectors for transformation into American chestnut embryogenic cultures. A unique *Pme I* site is present to facilitate substitution of promoters and for insertion of multi-gene constructs. Unique restriction sites to permit substitution of alternate selectable markers border the *npt II* gene with the associated Ubiquitin-10 promoter and NOS terminator. Triangles indicate T-DNA borders and an arrow indicates the region of T-DNA transfer.

Reporter gene vectors were constructed containing an intron-*β*-glucuronidase (GUSi) gene (*pFHI-GUSi*), a green fluorescent protein gene (*pFHI-GFP*), and a GUS-YFP fusion (*pFHI-GUSiYFP*). Each of the reporter gene vectors was transformed into embryogenic chestnut cultures and assayed for expression. PCR-based analyses of transgene integration indicated stringent selection with less than 1% escapes and PCR-positive embryogenic tissues exhibited high levels of reporter gene expression as determined by GUS assays and *in vivo* fluorescent imaging of green and yellow fluorescent proteins.

To date, fourteen candidate genes from Chinese chestnut, identified by comparative transcriptomics of *Cryphonectria*-inoculated American and Chinese chestnut, and two heterologous genes have each been cloned into the *pFHI-03* vector and transformed into American chestnut embryogenic cultures. Early screening methods were developed to identify transformed lines for each of the candidate gene vectors. Total genomic DNA was isolated from 5-10 mg of embryogenic tissue and analyzed by PCR using vector-specific primers (Figure 2). The approach reduces the transformation to screening interval from several months to six weeks and minimizes the number of cultures that are maintained to achieve the target of 40 translines per vector.

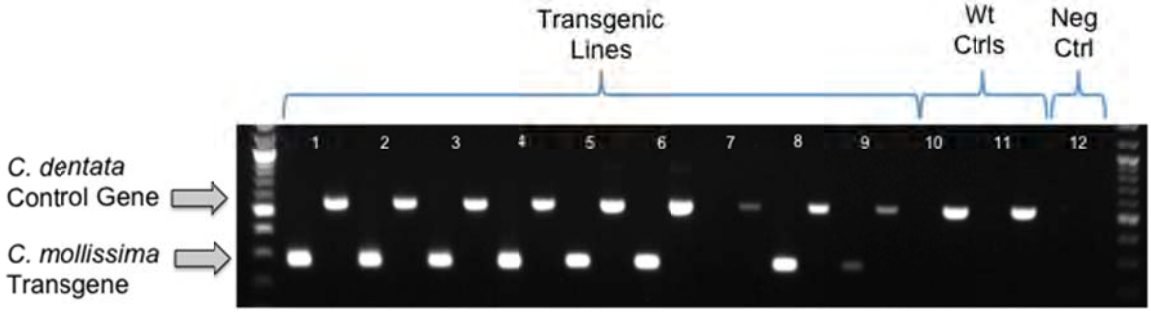

Figure 2. An example of results from PCR screens of transformed embryogenic chestnut tissues. Total genomic DNA was isolated from 5-10 mg tissue samples. DNA from each embryogenic line was assayed by PCR for the Chinese chestnut transgene (1-9). A second PCR reaction assays for an endogenous American chestnut gene was used as an internal control. Wild type American chestnut samples (10 and 11) were positive for the endogenous gene and negative for the transgene. No-template reactions were included as negative controls (12).

Following conversion to somatic seedlings and regeneration of plants, the materials are re-assayed to confirm transgene stability. Independent lines for each candidate gene and clonal materials derived from each line are evaluated to confirm transgene expression. Regenerated plantlets are transplanted into potting mix, then successively moved from growth chambers to the greenhouse and then into the nursery. Potted plants from the greenhouse containing candidate genes for *Phytophthora* resistance genes are subjected to *Phytophthora* screening. Plants with candidate genes for blight resistance will be grown in the nursery for two to three years until they reach a stem diameter suitable for blight resistance screening. Correlations between resistance levels, individual candidate genes, and gene expression levels will be examined.

Acknowledgements

The research was supported by funding from the Forest Health Initiative. We are grateful to ArborGen LLC for providing the binary vector pVWR-31.

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CHALLENGES OF FLUORESCENT TECHNOLOGY USE IN PLANT SPECIES

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Many new technologies are revolutionizing the study of genetics impacting both research and practical application. Use of fluorescence to monitor various reactions is a recent technology increasingly being used both *in vitro* and *in vivo.* We recently cloned a poplar metal-transport protein and modified it by adding red and cyan fluorescing proteins to the N- and C- terminal ends of the native protein. Subsequently, the protein was successfully expressed in two plant species including the hybrid poplar clone INRA 717-1B4 (*P. tremula* x *P. alba*) and *Arabidopsis thaliana*. The intent of this process was to create a bio-sensor to monitor heavy metals. After gene expression and fluorescence was confirmed, several challenges were identified when using fluorescence in plants. These include auto-fluorescence, differing cell physiology among tissues, physical location of the fluorescence, and changes of three-dimensional tissue aspects. Practical recommendations for use of this technology will be discussed.

COMPARATIVE GENOMICS OF ENVIRONMENTAL STRESS RESPONSES IN NORTH AMERICAN HARDWOODS

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The increasing incidence of introduced exotic pests, diseases and invasive plants, combined with climate change and forest fragmentation, threaten the sustainability of our forest ecosystems. The eastern hardwood forests are complex biological systems, covering over 400 million acres of bottomland and riparian sites, major watersheds, mesic sites and upland xeric sites. These forests provide habitat and food for wildlife, stabilization of riparian zones, long-term carbon sequestration and other essential ecosystem services as well as wood and biomass products for human use. Currently, few genomic resources are available for use in studying the consortium of hardwood species that compose the eastern forests. An interdisciplinary team are working together to develop new genomic resources for important species that represent the major taxonomic groups of eastern hardwood trees, from the oldest to more recently evolved, including yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), honey locust (*Gleditsia triacanthos*), northern red oak (*Quercus rubra*), black walnut (*Juglans nigra*), sugar maple (*Acer saccharum*), blackgum (*Nyssa sylvatica*), and green ash (*Fraxinus pennsylvannica*). The project will produce sequence databases for expressed genes, genetic markers, genetic linkage maps, and reference populations This will provide lasting genomic and biological resources for the discovery and conservation of genes in hardwood trees for growth, adaptation and responses to environmental stresses such as drought, heat, insect pests and disease.

GENOME SEQUENCE-ENABLED IDENTIFICATION OF AVIRULENCE GENES IN THE FUSIFORM RUST FUNGUS

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Cronartium quercuum f.sp. *fusiforme* (*Cqf*), the causative agent of fusiform rust disease has been damaging southern pine forests for decades. A complete *Cqf* genome sequence is currently under assembly at the Joint Genome Institute that will greatly facilitate identification of avirulence genes. Amerson and colleagues (manuscript in prep) have mapped 9 resistance genes (*R* genes) in loblolly pine suggesting that at least 9 corresponding avirulence genes (*Avr* genes) should exist in the fungus. The resistant reaction (i.e., no gall condition) requires the match of at least one *R* gene with its corresponding *Avr* gene for each spore that challenges the tree. Precise identification of these avirulence genes will provide the most accurate markers for measurement of allelic frequencies in various geographic locations. Frequency measurements can be used to guide selection of the most appropriate resistant pine genotypes. These kinds of preventive measures can now be realized more quickly with the aid of the *Cqf* genomic sequence. For example, the avirulence gene, *Avr1*, is known to specifically interact with the *Fr1* resistance gene. This avirulence gene exists within an 8.62 cM interval (about 0.3% of the genome), defined by 14 DNA markers: six Random Amplified Polymorphic DNA (RAPD) and eight Amplified Fragment Length Polymorphism (AFLP). The DNA fragments amplified by these markers have been sequenced and, for the AFLPs, the opposite parental allele has also been sequenced. Genomic sequence scaffolds will be bioinformatically screened with these fragment sequences to zero in on the target locus. Once identified, the locus can be fine mapped to pinpoint the *Avr1* gene. As additional markers are found to be linked to the other avirulence genes, their identification can proceed more easily using this method.

THE *CRONARTIUM QUERCUUM* F. SP. *FUSIFORME* GENOME PROJECT

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Cronartium quercuum f.sp. *fusiforme* (*Cqf*) is the causative agent of fusiform rust disease of southern pines. A complete *Cqf* genome sequence is currently under assembly at the Joint Genome Institute (JGI) that will identify all of the genes in this important forest pathogen. One biological objective of the project is to gain new insights into the relative number and nature of genes involved in *Cqf* host alternation, i.e., the requirement that particular spores produced on oaks can only infect pines and *vice versa*. Another objective is to identify virulence/avirulence genes so that managers can select appropriate genotypes for plantation establishment based upon allele frequencies in the local rust population. *Cqf* genome size was estimated by flow cytometry to be ~90 Mb (Anderson et al. 2010). For genome sequencing and to facilitate genome annotation, materials were collected for DNA and RNA extraction. DNA from the reference genotype was collected from pycnial droplets pooled from a single gall infected in the field near Saucier, MS. The sample contained single alleles only (i.e., there was no evidence for a mixture of genotypes) at six SSR loci. This suggests the sample is a haploid representation of the *Cqf* genome, which should aid assembly because there is no allelic variation in the sample. RNA was extracted from aeciospores (from pine), the hymenial layer from which pycnia were being produced in the fall (from pine), the hymenial layer from which aecia were being produced in the spring (from pine), basidiospores (from oak), teliospores (from oak) and diseased leaves containing vegetative mycelium, teliospores and basidiospores (from oak). Transcript assembly was performed to identify messenger RNAs encoded by *Cqf* genomic loci, and will be used to complement *ab initio* gene identification in the genomic assembly. BLAST analysis provides an estimate of host transcriptome representation in the transcript assembly, and generates an early glimpse of the *Cqf* "parts list."

ASSESSMENT OF GENETIC VARIABILITY IN RESISTANCE TO BROWN SPOT NEEDLE DISEASE IN LONGLEAF PINE: ANALYSIS OF PERFORMANCE IN TEST CROSSES

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Brown-spot needle blight caused by the fungus *Mycosphaerelia dearnessii* (formerly *Scirrhia acicolia* (Dearn.)) is the most debilitating disease of longleaf pine (*Pinus palustris* Mill.). This pathogen attacks seedling foliage tissue causing cell death in sections of needles, or in severe infections, throughout entire needles. As a result, seedling vigor is reduced leading to delayed emergence from the grass stage, and in cases of heavy infection, seedling mortality. Given the serious negative impact on longleaf pine regeneration in areas where this disease is chronic, there has long been interest in mitigating its effects through resistance breeding (Kais 1975). Previous research has shown that genetic variability in susceptibility exists within extant longleaf pine populations and that breeding for resistance is likely to be successful (Derr and Melder 1970; Snyder and Derr 1975).

As a means to facilitate early screening of seedlings for susceptibility to this blight disease, artificial inoculation methods have been introduced for use in greenhouse environments (Kais 1975). If shown to produce resistance readings that correspond well with field observations, this methodology will be an indispensable aid to selecting families and possibly individual trees with improved resistance. In this report, we present preliminary findings from research designed to obtain additional information about genetic variation in resistance to this disease.

Materials and Methods

Progeny of 56 families produced by mating four male parents with 14 female parents in a factorial mating design were evaluated in greenhouse tests as well as at two high disease-hazard field sites, one on the Harrison Experimental Forest (HEF) in Harrison County, MS and the other on the Palustris Experimental Forest (PEF) in Rapides Parish, LA. Trees chosen for use as male parents included two that in earlier tests produced progeny with relatively high resistance to brown-spot disease as well as two that were observed to produce more susceptible progeny. The sample of female parents contained trees found to yield progeny spanning a range of resistance levels varying from moderate to high.

Artificial inoculations were administered under greenhouse conditions using techniques similar to those described in Kais (1975) and Lott et al. (2001). Two separate inoculation experiments comprised of seedling progeny were carried out in adjacent sections of a single greenhouse. Seedlings in one experiment were inoculated with spores cultured from longleaf needles collected from PEF, whereas seedlings in the second experiment were inoculated with spores from HEF needles. Each experiment consisted of 24 replications made up of seedlings from the entire complement of 56 full-sib families randomized in single tree plots. Following inoculation

and a full growing season in the greenhouse, surviving seedlings were transplanted to the field sites using the same experimental design employed in the greenhouse — seedlings inoculated with PEF spores were transplanted to PEF and seedlings inoculated with HEF spores were transplanted to HEF. Prior to transplanting, diseased foliage was removed from each seedling.

In this report we summarize results for brown-spot disease damage to seedling in the greenhouse experiments three months post-inoculation and brown-spot disease damage ratings and total height at the field sites after two and five growing seasons, respectively. Severity of brown-spot disease damage to trees was visually estimated as percent of total needle area killed by the fungus.

Results

Foliage damage scores were transformed using the logit function prior to statistical analysis; however means and standard deviations are presented here in terms of the untransformed scale. Generally, light to moderate disease damage was observed in the greenhouse tests (GH) (Figure 1); nevertheless mean damage to seedlings exposed to the PEF inoculum (15.5%) was 20 percent less than the mean value for seedlings treated with HEF inoculum (19.4%) ($p < 0.0001$). Such a difference suggests that the two inocula differ in pathogenicity toward the longleaf pine population that was the focus of this study. After two growing seasons, damage observed in both field tests (FY2) was higher than in the greenhouse tests (Figure 1). It is worthy of note however, that the difference was clearly much greater for the HEF test. Mean foliage damage was 154 percent higher at this field site than in the PEF test (48.8% vs. 19.2%). This large difference ($F = 15.969$, $p < 0.0001$) may have resulted from a number of factors, including differences between the sites in pathogenicity of their respective pathogen populations, in fungal spore loads, and possibly in weather conditions conducive to spore dispersal. Regardless of the cause, these experiments provided the opportunity to study family performances under both endemic and epiphytotic conditions.

Narrow-sense heritability values for damage scores estimated from greenhouse data were low for both inoculum sources ($h^2 < 0.1$, Figure 2). Nonetheless, evidence was detected for variation among male-by-female family effects ($p < 0.07$ for PEF inoculum, $p < 0.0001$ for HEF inoculum), which suggests, modest nonadditive genetic effects are involved in protecting seedlings from disease damage. A different but somewhat similar pattern was found for narrow-sense heritability for damage estimates made after two field seasons. At the PEF site, the estimated value ($h^2 = 0.25$) implies presence of moderate additive genetic variance for resistance to brown-spot disease damage, whereas the estimate for the HEF site ($h^2 = 0.09$) was much lower (Figure 2) suggesting that less additive genetic variability is present in this trait for conditions existing at this location. It is conceivable that the high levels of disease damage observed at HEF (Figure 1), resulted in reduced expression of genes that influence inhibition of disease spread. Even so, much like inferences drawn from the greenhouse tests, F tests for the presence of male-by-female interaction effects ($p < 0.003$ for PEF, $p < 0.07$ for HEF) indicate that nonadditive genetic effects are involved in limiting disease development.

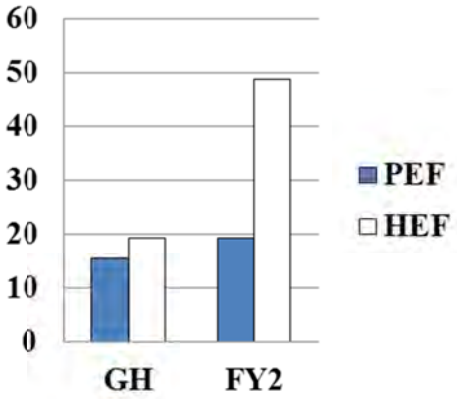

Figure 1. Means for percent foliage damaged.

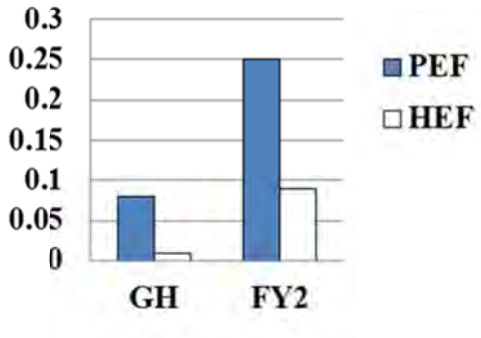

Figure 2. Narrow sense heritabilities for foliage damage scores.

Although there has long been awareness that increasing levels of brown-spot disease leads to decreasing tree height at intermediate stand ages (Boyer 1972), little is known about height growth decline in young stands. From a preliminary examination of our data, we noticed tree height variation also appears to decrease as foliage damage increases. Rates of decline for mean heights and standard deviations in trees at age five with increasing foliage damage at age two are illustrated in Figure 3. Similar patterns of growth loss and decreasing standard deviations were observed at the two test sites in our study. Mean height growth falls rapidly as foliage damage increases from ≤ 10 percent to 30-40 percent, then declines less steeply as foliage damage continues to climb. Standard deviation values drop less sharply, demonstrating that changes in this statistic are not a direct reflection of factors affecting the decline in mean height.

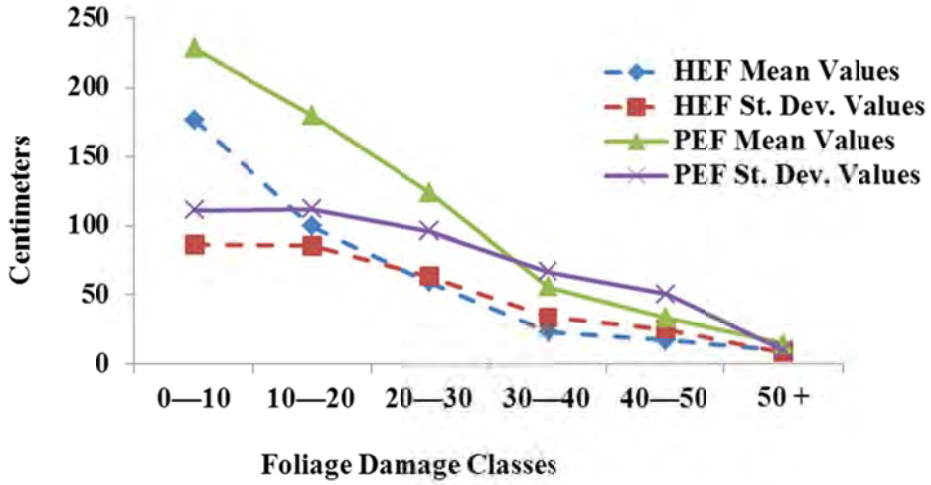

Figure 3. Changes in summary statistics for tree height at age five as foliage damage increases in two year old seedlings.

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QUATITATIVE GENETICS OF RESIN DEFENSE IN LOBLOLLY PINE

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Oleoresin that flows from the stem of pine trees is the primary defense against bark beetles and a potential source of bioenergy. We estimated the heritability of resin flow and chemical composition with clonal trials. We sampled oleoresin from 7600 individuals representing ~1000 loblolly pine genotypes derived from a partial diallel of 43 parents and 70 full-sib families that were clonally replicated at 3 sites in Georgia and Florida. We determined the dry mass of resin that flowed from a $1cm^2$ wound in the stem over 24 hours and used fourier transformed infrared spectroscopy (FT-IR) as a high-throughput method to assess wet oleoresin chemical composition. Oleoresin chemical composition was under stronger genetic control (α-pinene H^2 = 0.71) than resin mass (H^2= 0.17). Genotype x site interaction was minimal for resin chemical composition (r_B>0.95) and modest for resin mass (r_B=0.71). This work sets the stage for future research on the genes that control resin-related traits and breeding for enhanced resistance to bark beetles.

INVASIVE PESTS OF TREES - EFFECTIVE SOLUTIONS

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Arborjet® was developed by arborists for arborists in order to effectively manage and control the many exotic and native insect pests and diseases threatening our natural and urban forest today. Arborjet is the leader in tree injection technology and this presentation will cover effective control of exotic invasive insect species including, pine beetles, cone worm and borers using Arborplug™ and micro infusion technology. Arborjet methodology delivers insect/disease control products and micro-nutrients directly into the trees vascular system (xylem). Arborjet methodology, control products and trial data will all be covered in this presentation.

ESTABLISHING RESTORATION SEED RESERVES IN NATIONAL FOREST SYSTEM SEED ORCHARDS

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The role of the nine National Forest System seed orchards in the Southern Region is to support reforestation and restoration programs on the region's national forests. Traditionally, the emphasis of the seed orchards has been with commercially important tree species. Recently, emphasis is shifting to gene conservation aspects of restoration of ecologically important imperilled tree species. The main threats facing these species are shrinking ranges and declining populations that result, in part, from global climate change. Many factors influence the ability of forest species to adapt to changing conditions, but our focus is on the genetic factors affecting adaptive traits. The challenge is to ensure that imperilled species in national forests are managed to maximize their genetic adaptability to changing environments. So how can National Forest System seed orchards best support the response to this challenge?

Our basic concept is to establish seedling seed orchards that can produce seed with sufficient genetic diversity to impart to restored populations an ability to adapt to rapidly changing environments. When properly designed, the seed product from these "restoration seed reserves" (RSRs) should improve the resiliency of restored populations enough to avert extinction or extirpation of priority species in southern national forests. This model does not involve traditional genetic improvement, but rather involves enhancing adaptive potential of deployed germplasm. Although traditional tree improvement has long sought to maintain genetic diversity while improving marketable traits for a few commercial species, we are advising something different by developing a program for the genetic management of broader range species that have ecological value. Therefore, RSRs should rely not on artificial selection for specific traits or genotypes, but on managing gene flow among populations to increase their adaptive genetic diversity (Kramer and Havens 2009). A guiding principle in restoration plans that use seed from these RSRs will be to let natural selection do the heavy lifting and produce well-adapted tree populations. The supporting mission of RSRs, therefore, will be to supply "restoration ready", high quality, and genetically diverse seed for imperilled species management.

Twelve priority tree species and groups have been identified for RSR establishment: Atlantic white cedar, red spruce, Table Mountain pine, American chestnut, Carolina and Eastern hemlocks, seven threatened and endangered oaks, several ashes, butternut, longleaf pine, pitch pine, Fraser fir, September elm, yellow buckeye, and Ozark chinquapin (Crane 2011). These species were selected from a ranking of 140 southern species evaluated by the ForGRAS model for various risk factors associated with changing environments in the Southern Appalachian Mountains (Potter and Crane 2010).

We recommend harvesting at least 200 seed from each of 200 trees, *i.e.*, half-sib families, distributed across 20 to 40 separate populations. Mother trees should be spaced at least 100 m

apart, as the collection area permits. The target of 200 trees is maintained regardless of the number of populations sampled. Therefore, collect seed from 10 to 5 mother trees from 20 to 40 populations, respectively. As reproductive isolation increases among populations, then sample more populations, with fewer trees each. Collections from immediately imperilled or rare species could be extended to as many as 50 populations. However, that many distinct populations may not be available for a species in decline. In such cases, we recommend collecting from all available populations. This sampling design will capture virtually all alleles across the extent of the sampled range (Lawrence et al. 1995; Gapare et al. 2008). While a more comprehensive collection target might include 250 to 300 mother trees, our prescribed number of 200 will suffice when resources are limited.

A general RSR design for a species is as follows: germinate enough seed to establish twelve seedlings for each family, plant the seedlings in 4 x 3-tree family block plots, then at maturity evaluate trees for seed production and rogue each plot to one tree. This plan requires no progeny testing or family selection. Individual tree selection criteria are limited to flowering synchrony and fecundity. Selection for disease and pest resistance should be incorporated into a traditional tree improvement program, if warranted for a species. The result is 200 seed production trees in an RSR. Allocation of family plots in the orchard is not randomized, but rather is optimized to manage gene flow (cross-pollination) among families and groups. One type of optimized RSR design could maximize genetic diversity in the seed product by maximizing pollen exchange among populations. An alternate design could preserve ecotype traits by promoting pollination among populations whose families are planted in proximity. Orchard design optimization software is available that can weight or penalize family allocation by factors such as seed zone, ecotype, population, or kinship. (Fernández and González-Martinez 2010). We recommend establishing a species' RSR under the same or similar climatic and soil conditions found at the intended restoration sites or at least within the same seed zone, if known. To ensure a continuous supply of seed, successional RSRs should be established from new seed collections in anticipation of an eventual decline of RSR productivity or imbalance of family representation.

The RSR concept is similar to the tree breeding strategy of multiple origin polycrossing proposed by Ledig and Kitzmiller (1992) as a means to increase population heterozygosity in gene conservation programs. Broadhurst et al. (2008) proposed a related concept for seed transfer, "composite provenancing", to enhance genetic diversity in restoration populations. While rare, such forms of genetic blending can result in outbreeding depression or loss of local adaptation for some species, however, southern trees typically are generalists and not highly locally adapted (Schmitdling 2001). We maintain that the conservation paradigm of local-seed-is-best is a precautionary approach appropriate only when there is an abundant seed supply for direct seed transfer and when local populations are genetically diverse enough to endure throughout current and predicted ranges. However, if local seed is available from only small, isolated, inbred populations, then there may not be sufficient genetic diversity available to sustain local populations or maintain the species in the face of climate change. In such cases, augmenting the species' genotypic diversity with a variety of alleles and crosses from different populations could provide the necessary adaptive potential for forest restoration and sustainability.

Various options can mitigate potential risk in RSRs and species restoration plans. The most obvious is to establish duplicate RSRs for each species to minimize the chance of catastrophic loss from fire, severe weather, or even land development. For some species, this may require establishing additional seed orchards or seed production areas on national forest ranger districts. A second RSR or seed production area could be established in a different climatic zone or different seed zone; although there is no clear evidence this would improve restoration success over the expected life of the RSR. Another option, if a precautionary approach is desired for some species, is to establish supplemental seed production plot(s) dedicated to "local seed" production. Using different seed production plots with different rates of gene flow among sampled populations is an adaptable strategy because seed from different production plots could be mixed in desired ratios for deployment. Spare seed collected from mother trees could be archived in cold storage, though seeds of some species quickly lose viability in storage.

In summary, RSRs allow managed gene flow among sampled populations that is tailored to the restoration needs of the target species. The key features are that RSRs: 1) avoid inbreeding by having no clones or siblings, 2) limit selection to essential seed production traits, 3) manage gene flow with an optimized planting design, and 4) can increase adaptive diversity of restoration populations beyond what is currently available from native seed supplies.

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CLIMATE CHANGE AND GEOGRAPHIC VARIATION IN THE SOUTHERN PINES

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Climate change has been occurring for eons, and plant species have always responded by migrating to areas of more favorable climate or evolving in place. The rapid pace of climate warming that is now occurring may not allow enough time for the changes to take place. For species that are commonly planted, such as the southern pines, projected climate change can be incorporated into planting efforts by utilizing known geographic variation to account for that variation. Geographic variation in the southern pines is reviewed, and seed-movement strategies discussed.

GENETIC VARIATION IN A LONGLEAF PINE POPULATION: A LONG-TERM FIELD STUDY OF A 13-PARENT DIALLEL

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Genetic and environmental effects on growth and survival were quantified in a population of longleaf pine (*Pinus palustris* Mill.) using a 13-parent diallel cross growing over 40 years on two sites in southern Mississippi. This long-term field experiment is unique for longleaf pine, a tree species of renewed interest for ecosystem restoration and plantation forest management in the southern United States.

Materials and Methods

The genetic foundation of this experiment consists of progenies produced by making all possible crosses among 13 parent trees in a diallel mating scheme. The parent trees were randomly chosen from a natural, open-grown longleaf pine stand located on the Harrison Experimental Forest (HEF) near Saucier, Mississippi. Of the 169 possible crosses, 143 were planted in 1960 in two replicated field trials, each located on the HEF about 2 miles apart. Of the 78 half-diallel, full-sib crosses, 76 were established in field tests. Each site was established in a randomized complete block design experiment composed of eight-tree family row plots arranged in four replications. Growth and survival data were collected for all trees at various stages of stand development, including ages 3, 7, 17, 30, and 40 years.

Quantitative genetic analyses were conducted and reported on for ages 1 through 7 years, by Snyder and Namkoong (1978). Further analyses of genetic parameters, including age 17 year results, were reported by Rousseau (1980). Later ages including age 40 years were analyzed and reported by Stine et al. (2001). Measurements for ages 3, 7, 17, 30, and 40 years are analyzed for this report. Only families that had greater than two replications per test site were included in this analysis. Of the 76 full-sib crosses, 66 families met this criterion. In this analysis, ASReml software was used to generate genetic variance components of a mixed model (Gilmour et al. 2002).

Results and Discussion

The strong genetic basis of the experiment, coupled with superb silvicultural management of the sites, allowed us to successfully partition the genetic and environmental components of variation in various growth and survival traits. We found substantial genetic variability in growth and survival among these longleaf pine families. Family means for individual-tree volume ranged from 12.7 to 24.9 cubic feet (0.36 to 0.70 cubic meters) at age 40 years.

For later ages (≥17 years) growth traits, non-additive genetic (specific combining ability, SCA) variance was high, relative to additive genetic (general combining ability, GCA) variance (Table 1). Similarly, the ratio was high for tree survival trait at age 40 years (Table 1). The variance component estimates along with their standard errors are reported in Table 2. The relatively large proportion of non-additive genetic variance after age 7 years is noteworthy and is consistent with previous reports (Rousseau 1980; Stine et al. 2001). However we are reporting larger non-additive to additive genetic variance ratios for some traits. Our current analysis includes a larger sample of families than used in the analyses of Stine et al. (2001), because of our less stringent restriction on numbers of trees required per family, an additional earlier age (age 3 years), and we have used different computational and analytical methods. It remains to be seen whether this stronger non-additive effect holds up in further analyses, but clearly we are seeing a trend of higher SCA variance versus GCA variance starting after stand closure and continuing to mature stand ages.

Broad implications of these results for longleaf pine breeding and species conservation can be stated as follows. First, breeding programs for longleaf pine should not ignore specific combining ability (SCA) in developing production and breeding populations. Second, substantial genetic variation in growth and survival were found among non-selected parent trees in a natural stand within a small area (80 acres), indicating that much genetic variation for important traits exists in natural populations of longleaf pine.

Table 1. Ratios of non-additive to additive genetic variances for the longleaf pine diallel experiment for growth and survival across different ages. Data suggest that by age 17, non-additive genetic variance is generally large compared to additive genetic variance.

Trait	Age 3	Age 7	Age 17	Age 30	Age 40
Height	0.0	0.22	2.1	- -	0.80
DBH	- -	0.38	1.32	1.27	3.21
Volume	- -	- -	1.35	- -	2.23
Survival	- -	0.08	0.45	0.27	2.76

Table 2. Genetic variance components (w/ std. errors), and ratios of non-additive (SCA) to additive (GCA) genetic variance at ages 17 and 40 in the longleaf diallel experiment.

Trait	SCA	GCA	Ratio, SCA/GCA
		Age 17	
Height	78.25 (30.8)	36.5 (22.4)	2.1
DBH	5.39 (2.02)	4.08 (2.07)	1.32
Volume	18.07 (7.81)	13.40 (7.02)	1.35
Survival	0.035 (0.037)	0.076 (0.040)	0.45
		Age 40	
Height	95 (70.0)	118.5 (61.2)	0.80
DBH	16.13 (5.76)	5.03 (3.24)	3.21
Volume	215.25	96.5 (59.3)	2.23
Survival	0.140 (0.053)	0.051 (0.031)	2.76

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HYBRIDIZATION IN NATURALLY REGENERATED SHORTLEAF PINE NEAR ARTIFICIALLY REGENERATED STANDS OF LOBLOLLY PINE

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Shortleaf pine (*Pinus echinata* Mill.) is an important timber species found throughout the southeastern United States. The species is known to hybridize with loblolly pine (*Pinus taeda* L.), another very important timber species in the southeastern United States. While the two species often occur together, loblolly pine generally occurs on more mesic sites, and shortleaf pine is generally found on more xeric sites. In the Mid-South, the natural range of loblolly pine ends at the northern edge of the upper west Gulf Coastal Plain; as a result, shortleaf pine is the only naturally-occurring pine in the Ouachita and Ozark Mountains, where it often forms pure pine-dominated stands especially on southern aspects. The transition from mixed loblolly pine and shortleaf pine stands to shortleaf pine stands without loblolly pine is occasionally patchy. Additionally, timber companies prefer to plant loblolly pine in their plantations, which may be outside of the natural range of the species.

Factors leading to introgression in loblolly pine and shortleaf pine may include the distance between stands, or the common condition in which both species are naturally found together in stands in the upper west Gulf region. In the present study we used microsatellites to measure levels of hybridization and introgression in naturally regenerating shortleaf pine stands in the Caney Creek Wilderness Area located on the Ouachita National Forest in west-central Arkansas. This area is allopatric by about 40 kilometers relative to loblolly pine. However, extensive plantations of loblolly pine have been established in this area over the past four decades on private lands managed intensively for timber and fiber productivity in support of local forest industry.

Materials and Methods

Current-year leaves were collected from four locations in the Caney Creek Wilderness Area, Polk County, Arkansas, which is part of the Ouachita National Forest. Each collection location was on the corner of an approximate rectangle about 32 km from east to west and 24 km from north to south. Collection sites were thus labeled northwest (NW), southwest (SW), northeast (NE), and southeast (SE). Twenty-five possible parent trees—trees that were at least 30 years old and labeled NWp, SWp, NEp, and SEp, depending on the source location—were sampled from each location, and 100 naturally regenerated seedlings/saplings, which were labeled NW, SW, NE, and SE, were also sampled from each location. The DNA was extracted from leaf tissue through cutting the leaves into small pieces and then using the Qiagen DNeasy 96 Plant Kit (Qiagen, Velencia, CA).

Twenty-five microsatellite markers previously confirmed to be polymorphic in both speices were used in this study. Three primers for these markers were used during PCR, two that flanked the

short sequence repeat region and one primer labeled with a fluorophore. All PCR products were scored using a LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE).

General population genetic analyses were performed with the software GenAlEx 6.3, which calculated Φ_{PT}, Nei's genetic distance, the inbreeding coefficient (F_{IS}), expected heterozygosity (H_E), observed heterozygosity (H_O), and Hardy-Weinberg Equilibrium statistics. Structure version 2.3.2 was used to determine hybrid character of individuals. We set population number k to 2, which represents the two species analyzed in this study. Hybrids were reported when predicted genome proportion levels (*Q*) were between 0.9531 and 0.0469, about what is expected for trees in an F1 cross or a first through third backcross generations. In order to test whether the proportion of hybrids in the sapling populations was different from the proportion of hybrids in the parent populations, a X^2 test was performed, using the proportions of individuals that were assigned to each hybrid category: F1s and loblolly pine backcrosses, shortleaf pine backcrosses, and shortleaf pines.

Results and Discussion

The estimate of population differentiation (Φ_{PT}) was calculated across all 8 populations to be 0.064, which is normal for a wind-pollinated forest tree and is in agreement with previous measures for the species (Stewart et al 2010). The correlation coefficient (R^2) for geographic distances and genetic distances of the populations was 0.103, which is insignificant (p= 0.057). Average expected heterozygosity (H_E) for all populations was 0.514. For the parent populations, average H_E was 0.497, and for the sapling populations, average H_E was 0.531. Average observed heterozygosity (H_O) for all populations was 0.422, while average H_O for the parent populations was 0.409, and average H_O for the sapling populations was 0.435. The mean inbreeding coefficient (F_{IS}) was 0.176, indicated little inbreeding. F_{IS} for the saplings (0.169) was similar to F_{IS} for the parents (0.189). Of the 25 markers used in this study, 5 of them passed the Hardy-Weinberg Equilibrium test, showing that the population as a whole is in transition.

In all sample sites, the measured number of hybrids decreased, but chi-square tests showed that any differences were not statistically significant. Correlations for different measures for relationship between the geographic distance to the nearest loblolly pine plantations and levels of hybridization were all insignificant. However, all trend lines were negative, which would be expected, as trees more distant from the plantations should have less hybridization than trees that are closer to the plantations. The relationship between the distance from the nearest loblolly pine plantation versus average *Q* value among the saplings was $R^2 = 0.3060$ ($p = 0.447$). That relationship among the parents was $R^2 = 0.4317$ ($p = 0.343$). The relationship between the nearest loblolly pine plantation versus the average *Q* value among the combined populations was $R^2 = 0.3293$ ($p = 0.137$). The relationships between distance from the nearest loblolly pine plantation and the percent hybrids in each population was $R^2 = 0.3851$ ($p = 0.379$) among the saplings, $R^2 = 0.886$ ($p = 0.059$) among the parents, and $R^2 = 0.4768$ ($p = 0.058$) among the combined populations. The lack of correlation may be due to an insufficient number of sites sampled and the sites being too close together.

On average, the pines in this study had 2.50 leaves per fascicle with a standard deviation of 0.336, which is consistent with the common description of shortleaf pine having two or three leaves per fascicle. The average leaves per fascicle for each individual did not correlate with those individuals' structure values, either when all individuals were included (R^2=0.01) or when only hybrid individuals were included (R^2=0.06).

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GENETIC RESOURCE MANAGEMENT AND CLIMATE CHANGE: GROWING HEALTHY FORESTS FOR THE FUTURE

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Providing seed for operational reforestation and restoration has long been the principal focus of the U.S. Forest Service's Genetic Resource Management Program. Although this work will continue into the future, climate change predictions will require changes in the ways these needs are met.

The guiding principle for managing the genetic resources of National Forests has been through the use of local seed sources in reforestation and restoration. The advent of a rapidly changing climate, however, means that a new paradigm will be required to maintain healthy and productive vegetation on National Forests and to preserve at-risk species and populations. At a minimum, the current practice of relying on seed sources that were best suited to the past climate will need to shift to allow consideration of the source, or sources, of seed that will be best suited to predict future climates. In many cases, species and seed sources that may be optimal under climate change scenarios have not received adequate research or management attention and thus lack basic genetic information as well as sufficient representation in forest seedbanks. More aggressive gene conservation programs, especially ex situ seed collection, will also be needed for species and populations most vulnerable to climate change impacts.

At present, there is no generally applicable national guidance for incorporating climate change impacts into the management of National Forest genetic resources. In the spring of 2010, Forest Service and university geneticists convened to share background information and develop consensus for revising National Forests System genetic resource management guidelines. The goals of the meeting were to 1/ provide information on climate change scenarios and potential effects on vegetation and forest genetic resources, 2/ facilitate the interaction and exchange between climate scientists and geneticists to develop strategies for responding to climate change in Forest Service genetic resource management programs, 3/ identify genetic options for responding to climate change and its effects on vegetation and genetic resources, with an emphasis on the next 5-10 years, 4/ identify gaps related to information, resources, research, and tools needed to manage effectively within a changing climate. A whitepaper on key genetic issues, guidelines, and program gaps/needs in light of climate change is currently under development.

SCALING-UP TRANSGENIC AMERICAN CHESTNUT SOMATIC SEEDLING PRODUCTION FOR THE FOREST HEALTH INITIATIVE

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American chestnut (*Castanea dentata*) was once one of the most important forest trees of eastern North America, both ecologically and economically. With a range stretching from Maine to Alabama, it reached its greatest size and density on the ridges and benches of the Southern Appalachians, where it is estimated that one in four trees was an American chestnut. Huge volumes of American chestnut were harvested annually in the region, and the durable wood was used for construction of houses, barns, fences and furniture. The nutritious nuts were consumed by wildlife and people. The central role of chestnut in Appalachian life changed dramatically following the accidental introduction of the chestnut blight fungus (*Cryphonectria parasitica*) on Asian chestnut planting stock late in the 19th Century. The fungus is a necrotroph, entering any wound in the bark and producing a mycelia fan under the bark that kills the cambium and young xylem and phloem with oxalic acid and then consumes the dead tissue. Eventually, the stem is girdled, killing the tree. First documented in New York City, the blight spread southward at the rate of 200 miles every ten years, killing almost every American chestnut tree in its path. Since its appearance, multiple approaches to combat chestnut blight and restore the American chestnut to the forest have been attempted. These approaches have included application of fungicides, searches for naturally resistant American chestnut trees, mutation breeding via gamma irradiation of nuts, hypovirulence and breeding with resistant Asian chestnut species. While the initial attempt by the USDA to use this last approach ended in apparent failure, a more recent hybrid backcross breeding program initiated by The American Chestnut Foundation in the 1980s has made substantial progress toward producing hybrid trees with near-Chinese chestnut levels of resistance.

Another recent approach to production of blight-resistant trees involves application of *in vitro* propagation and genetic engineering. Laboratories at the University of Georgia (UGA) and The State University of New York-Environmental Science and Forestry (SUNY-ESF) have been conducting research on genetic engineering of chestnut for over 20 years. While these labs made substantial progress developing protocols for producing transgenic chestnut trees, production of trees with genes with potential anti-fungal activity lagged until recently, when significant new support for the research was provided by the Forest Health Initiative (FHI). FHI chose American chestnut as its first target for research in its mission to demonstrate the application of biotechnological tools to address forest health threats in the U.S. The application of in vitro clonal propagation and transgenics is part of a "braided" approach to bring the tools of biotechnology to bear on the chestnut blight problem, which also includes efforts in the areas of germplasm, breeding, genomics and gene discovery. In addition to biological sciences research, the FHI also includes teams focusing on social and environmental issues and on regulatory and legal affairs associated with biotechnology and forest health. As part of this effort, we are collaborating with scientists from multiple universities (SUNY-ESF, Penn State, Clemson), The

American Chestnut Foundation (TACF) and the USDA Forest Service to employ somatic embryogenesis (SE) for several project objectives. SE will be used to propagate blight-resistant hybrid backcross-derived material from TACF's breeding program for clonal testing. SE will also provide target material for testing candidate genes (CGs) from Chinese chestnut and heterologous sources that may provide resistance to the blight fungus and/or *Phytophthora*, which is a particular problem in the southern part of the range. With regard to transgenics, screening of hundreds of embryogenic cultures has already been conducted to identify a handful of "workhorse" culture lines that will be the main targets of transformation with all CGs. However, in order to be selected as a "workhorse" line, a culture line not only has to be "captured" and grow well in suspension culture, but has to successfully pass a number of other bottlenecks, including unambiguous sensitivity to selection agents and ability to produce high-quality somatic embryos and somatic seedlings following transformation.

New embryogenic cultures were initiated from several American chestnut full-sib and half-sib families from different parts of the range hybrid backcross material in 2009 and 2010. In 2009, over 9000 seeds were cultured, resulting in 64 new embryogenic cultures lines or an overall capture rate of 0.7%, while in 2010, over 8500 seeds were cultured, producing 107 new embryogenic cultures for an overall capture rate of 1.23%. A new germplasm agreement with TACF enabled culture, for the first time, of TACF B3F3 hybrid backcross material, resulting in capture of embryogenic cultures from 10 B3F3 families representing two lines of blight resistance. Copies of all embryogenic chestnut cultures were cryostored following our published protocol (Holliday and Merkle 2000). Once established, 2009 embryogenic cultures were screened for potential to produce somatic embryos and somatic seedlings and displayed a range of productivities. Some lines could produce over 100 well-formed somatic embryos per 0.5 g of starting material, following our published protocol for somatic embryo production from suspension cultures (Andrade and Merkle 2005), and some culture lines demonstrated conversion frequencies over 50 percent. Lines initiated in 2010 are currently being screened for somatic embryo and somatic seedling production potential. Culture lines capable of producing a minimum of 10 somatic seedlings are needed to test the concept of clonal testing of genotypes for blight resistance. In addition, information from these culture screens was used to choose three of the most productive lines to uses as "workhorse lines" for transformation with CGs.

Transformation of workhorse lines with new, modular CG and reporter gene constructs began in 2010, using the transformation protocol detailed in Andrade and Merkle (2009). At the start of the project, we projected that we could move only 2-3 CGs through the transformation/regeneration "pipeline" per year, based on the shaken flask-based embryogenic culture system we were using at that time. However, our recent adoption of airlift bioreactors for growing embryogenic suspension cultures, rather than shaken flasks, greatly accelerated production of embryogenic material for both somatic embryo production and *Agrobacterium*-mediated genetic transformation. Currently, sufficient new target material for transformation experiments can be produced every two weeks. This change allowed us to transform 12 CG constructs and 3 reporter gene constructs into embryogenic American chestnut cells to in 8-9 months. Transformation frequencies for some target lines have been very high, producing almost 700 putative transformation events per 50 mg of inoculated tissue of one target line. Over 5000 transgenic events in 3-4 backgrounds (target lines) have been captured. Somatic seedlings

carrying the first FHI CG, an anti-fungal peptide gene from the *Gastrodia* orchid, will be transferred to the greenhouse soon and somatic embryos with four more CGs are in production. Our goal is to generate 20 plant-producing transgenic events per CG, each capable of producing at least 10 transgenic trees to be screened in the field for blight and/or *Phytophthora* resistance. The first field planting of transgenic chestnuts produced at UGA was installed in May 2011. Over 100 transgenic chestnuts carrying the ESF39 synthetic antimicrobial peptide gene (Powell et al. 1995) were planted and some of these trees should be sufficiently large by summer 2011 to inoculate with the blight fungus for resistance screening.

Acknowledgements

The work reported here was supported by the Forest Health Initiative. We would also like to thank ArborGen LLC and the Institute of Forest Biotechnology for support, and The American Chestnut Foundation and the American Chestnut Cooperators Foundation for providing chestnut material for our research.

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SILVICULTURE OF VARIETAL LOBLOLLY PINE PLANTATIONS: EVALUATION OF SPACING AND SILVICULTURAL TREATMENTS ON GROWTH AND INTRACLONAL UNIFORMITY

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During the last decades, the efforts in genetic improvement have contributed to increase productivity of loblolly pine in the South (McKeand et al. 2003). Recently, there has been increased interest in developing varietal forestry. It has been reported that higher genetic gains can be achieved by using varieties, resulting in higher yield and phenotypically more uniform stands (Zobel and Talbert 1984). Also, it has been argued that the potential deployment of elite genotypes will require intensive levels of silviculture including site preparation, weed control, fertilization, and spacing.

Working at a variety level, the expected responses in growth may vary considerably due to site or silvicultural treatment differences. Stoval et al. (2010) found contrasting results for different loblolly pine varieties in response to fertilization, which were tested in the same site conditions. Some varieties showed positive response to fertilization, others did not response, and a few of them exhibited a negative response. On the other hand, increasing the genetic uniformity is not necessarily associated with an increase in the uniformity of growth or physiological traits (Aspinwall et al. 2011). In this report, we present some preliminary results of two-years trials located in two contrasted sites in the southern United States (Virginia Piedmont and North Carolina Coastal Plain), where the same genotypes were planted and managed using intensities of silviculture.

Materials and Methods

A study of varietal silviculture of loblolly pine was established in 2009 at two sites in the southern U.S. One site was located in the Virginia Piedmont at the Virginia Tech Reynolds Homestead Research Center. Soils at this site were well-drained Fairview series. This site previously supported a mixture of loblolly, Virginia and white pine stands that were harvested in 2007 and 2008. The second site was located in the North Carolina Coastal Plain at Bladen Lakes State Forest. Soils at this site were poorly drained Rains series. This site previously supported a loblolly pine stand. The study was a split-split plot design with two levels of silviculture (operational and intensive) as the main plots, six genotypes entries (1OP, 1CMP, 4 clones) as sub-plots, and three different planting densities (250, 500, and 750 trees per acre) as sub-sub-plot. At the Reynolds site, the experimental unit consisted of block-plots of 81 trees (in an arrangement of 9 by 9 trees) with 4 replicates. At the Bladen site the experimental unit was a block-plot of 63 trees (in an arrangement of 9 by 7 trees) with 3 replicates.

The site preparation at both sites was a chemical application, followed by a broadcast burning at Reynolds, and a V-blade bedded using a Savannah bedder at Bladen. The operational silviculture

consisted of a banded weed control during the first growing season, whereas the intensive silviculture included broadcast herbaceous weed control during year 1 and 2 and an application of fertilizer (150 lbs/acre N + 25 lbs/acre P) during the winter prior to the start of the second growing season. The genetic entries consisted of 1 open pollinated family (OP), 1 mass control pollinated family (CMP), and four varieties designated C1, C2, C3 and C4. The varieties C3 and C4 were selected as having a wide crown ideotype, whereas C1 and C2 were selected having a moderately broad crown.

Total height and crown width were measured during winter (January 2010 and January 2011). Crown width was measures twice, parallel and perpendicular to the planting row, and the average per tree was used for used in the analysis. The means and coefficient of variation was calculated at each experimental unit, which were subjected to analysis of variance to determine the sources of variability in growth and uniformity. The analyses of variance were done with the MIXED procedure of the Statistical Analysis System (SAS), version 9.2 (SAS Institute, Cary, NC, USA).

Results and Discussion

At the end of the second growing season, tree height was a 39% higher at Reynolds (Virginia Piedmont) than in Bladen (North Carolina coastal plain, Figure 1). The levels of the water table in the poorly drained soils at the Bladen site, which probably affected tree development at this stage. However, we anticipate that the growth will be greater at Bladen in the future when tree development increases and higher evapotranspiration rates decrease water table level. Moreover, a higher variability of growth was present in the plots at Bladen than Reynolds, with within-plot coefficient of variation of 41% and 26% in the second year, respectively. Microtopographic differences in soil drainage likely affect root growth and increase heterogeneity in growth across the landscape in the second year. At the Reynolds site, where the soils are well drained, there was not an increase in the plots variability across the years (Figure 1).

There was no significant effect of planting spacing and silvicultural treatments after 2 years. However, plots having intensive silviculture performed better in growth at the two sites. The averages for tree height at the second year were 23% and 7% higher with intensive silviculture than operational at the Bladen and Reynolds sites, respectively. The high variability in growth within the plots probably masked the differences in the silvicultural treatments at this age.

At the two sites, there were significant differences among genetic entries for all the variables. Means tree height for the varieties were significantly different from the OP and CMP families at both sites and years (Figure 1). Moreover, higher within-plot uniformity was found using varieties, supporting the hypothesis of varietal forestry in which increasing the genetic uniformity should increase the stand yield and uniformity. These results must be carefully interpreted because of the narrow genetic diversity tested. Increasing the genetic uniformity is expected a major genetic control of quantitative traits of interest such growth, and uniformity; however, there is some evidence contradicting this fact (Aspinwall et al. 2011). Although varieties had similar growth rates each site, variety 4 was more uniform (lower CV) at both sites, which highlights the importance of selection from this point of view.

There was a positive correlation between crown width and tree height, with values of 0.82 and 0.7 for Bladen and Reynolds site, respectively. Varieties had greater crown width values than families, but there were not differences between the varieties with different crown ideotype at this stage. Despite of the young age of the trees, our preliminary results showed that growth and uniformity was higher in plots containing varieties than families, and that differences are accentuated with intensive silviculture.

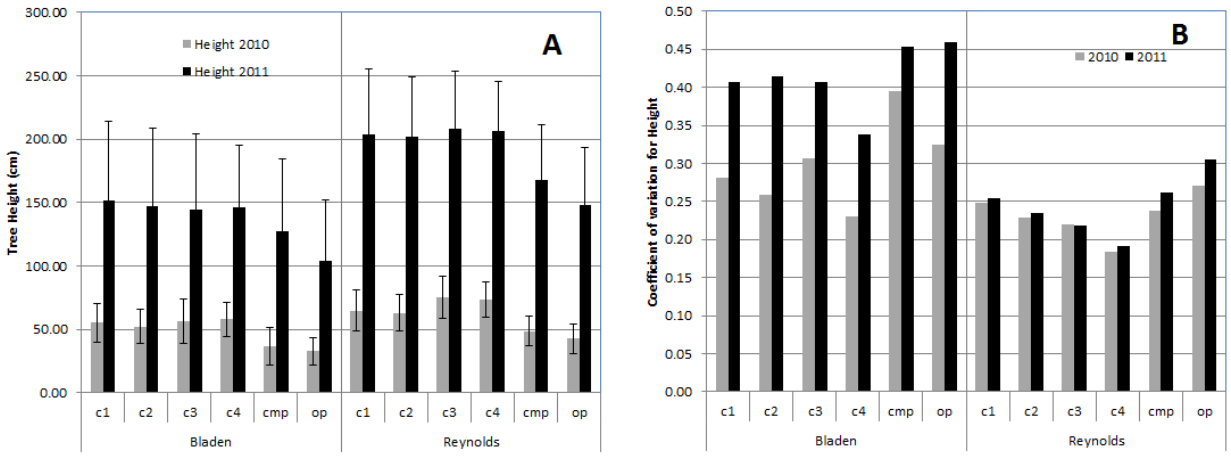

Figure 1. (A) Means tree height obtained per site and genetic entries during the first two years. Bars designate one standard deviation. (B) Coefficient of variation of each site and genetic entries during the first two years.

Acknowledgements

NSF Center for Advanced Forestry Systems; ArborGen Inc.; Weyerhaeuser Timberlands; North Carolina Forest Service – Bladen Lakes State Forest; Virginia Tech –Reynolds Homestead Forestry Research Center; Valor Florestal; Forest Productivity Cooperative; IPEF.

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SUN GRANT *POPULUS* FEEDSTOCK PROGRAM

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The focus of the project is to develop clones of either *Populus* species or hybrids for the Southeast, Pacific Northwest, Midwest, and North-Central United States. The initial step was to establish a series of consolidated clone trials where each of the four cooperators, which included ArborGen, GreenWood Resources, University of Minnesota, and Mississippi State University, provided 20 clones. This test series were established in 2010 and 2011 at five locations. The clones contributed by the University of Minnesota were primarily recently developed *P. deltoides* x *P. nigra* (DN) hybrids. The hybrid clones provided by GreenWood Resources incorporated a variety of species combinations including *P. trichocarpa* x *P. deltoides* (TD), *P. trichocarpa* x *P. nigra* (TN), *P. deltoides* x *P. maximowiczii* (DM). The ArborGen clones were primarily *P. deltoides,* but also included three TD hybrids. Mississippi State provided all *P. deltoides* clones. Growth and disease ratings are the two traits that will be examined prior to more intensive testing. Breeding began in 2011 and is aimed at producing both intra- and interspecific hybrids for use on more marginal sites. The parents selected for the initial stages of breeding include more recent *P. deltoides* and *P. nigra* selections. Other efforts in 2010 and 2011 include a *P. nigra* clone test, an open-pollinated *P. deltoides* progeny test and a series of permanent growth and yield plots. Future plans call for additional clonal screening trials, intensive clonal tests, and an increased emphasis on breeding efforts.

GENETICS OF PURE AND MIXED FAMILY PLOT YIELDS IN LOBLOLLY PINE

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Mixed and pure family plots of 10 half-sib families of loblolly pine (*Pinus taeda* L.) were planted in replicated experiments at two spacings on two sites in south Mississippi and Louisiana. Plots sizes were 70 trees (10 x 7 tree blocks) with three complete replications per spacing per site. Tree spacings were 1 x 2 and 2 x 2 meters (~2000 and ~1000 TPA, respectively) and individual tree measurements were made at ages 5, 10 and 15 years. Mixed plots consisted of paired families randomized in 75/25 and 25/75 ratios of trees planted as well as all families in randomized rows and all families in random mixes. By age 10 the wider spacing plots were yielding at or near to the closer spacing plots. Stem volume yield differences among treatments (family composition X plot type) appeared stronger at closer spacing (1 x 2 m) than wider spacing (2 x 2 m), and family composition was the strongest factor, over mixed vs. pure plots and ratios within mixed plots. Mixed family plots yielded higher than pure family plots, although family x mixture ratio and family x spacing were significant sources of variation. Some binary family mixes appeared promising for maximizing plot yield. From a genetic testing standpoint family row plots were more highly correlated with family pure plot yields than were non-contiguous plots.

THE EFFECT OF THE BLUP BREEDING VALUES IN GENOMIC SELECTION ACCURACY

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In tree and cattle breeding populations, pedigree errors have been estimated to be ~ 10%. Even minimal errors in pedigree can cause an underestimate of the additive variance and a decrease in the BLUP breeding value (BV) prediction accuracy. Traditionally, a relationship matrix (A) derived from the pedigree is used in mixed model prediction of BV (Henderson 1972). The A matrix has the expected relationship values between individuals (i.e. 0.5 for full-sib) but does not account for variation caused by Mendelian segregation among individuals (Mrode 2006). Simulations have demonstrated that the observed relationship matrix (ORM) derived from a panel of SNPs can be used to correct pedigree relationships. In addition, use of the ORM rather than the A matrix in the BLUP analysis of phenotypic data yields less biased estimates of the true heritability and more accurate predictions of BV.

Predictions of BV with traditional BLUP analysis from phenotypic data are the input for construction of prediction models based solely on genotypic information, genomic selection (GS) (Meuwissen et al. 2001). Posteriorly, BVs are correlated with GS-predictions to assess the accuracy of the GS models. Thus, bias in BVs and/or pedigree errors should decrease GS accuracy.

The objectives of this study are to construct the ORM from SNP data available from a small pine breeding population to first compare the accuracy on the BV prediction by using the original pedigree in BLUP and by using a pedigree corrected using the ORM. Second to determine the effect on the GS accuracy using these sets of BVs. Evaluate the effect of incorporating the ORM directly in the BLUP BV prediction. And to evaluate the effect on GS accuracy when using BV that have confounded non-additive effects.

The data correspond to a clonal population of loblolly pine phenotyped at six years for total height (HT) and genotyped with SNPs markers. The ORM was constructed based on a recently published method. BLUP analysis were performed in ASReml (Gilmour et al. 2006), and GS was performed using the RR-BLUP method (Meuwissen et al. 2001). Accuracies for BV predictions were obtained following Mrode (2006) and GS accuracy was calculated as the correlation between the BLUP-BV and the GS-BV (Goddard et al. 2009).

When compared to traditional BLUP with the original pedigree, using the ORM corrected pedigree increase heritability from 0.26 to 0.31, BLUP-BV accuracy from 0.80 to 0.85, and GS accuracy from 0.64 to 0.77. An additional increase in GS accuracy (3%) could be reached if the ORM is incorporated directly in the BV predictions by BLUP. Finally ignoring non-additive effects on the BV prediction by BLUP and using those BV in GS generate a considerable decrease in GS accuracy (33%).

IMPACTS OF SPACING AND GENETIC HOMOGENEITY ON GROWTH PATTERNS IN JUVENILLE LOBLOLLY PINE

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There is a lack of information on the effects of genetic homogeneity on individual stem- and stand-level characteristics for loblolly pine. With the increasing acreage that is planted from a specific genetic family, research is needed to compare differences in growth patterns across varying levels of genetic homogeneity. In this study, we wanted to compare stand uniformity and productivity among loblolly pine genotypes of contrasting inherent genetic homogeneity while incorporating two planting densities. To examine genetic effects on stand uniformity and productivity, we grew ten different genotypes (three open-pollinated families, three full-sib families, three clones, and one seed orchard mix variety) in a plantation setting for 5 years, at two different planting densities (436 and 218 tree per acre). At age 5, average volume of the most productive genotype at the low planting density was 147% greater than that of the least productive genotype. Furthermore, the high density planting yielded similar results with a gain of 145% over the least productive genotype. Annual volume ranks among the genetic entries were consistent at the high planting density. In contrast, the volume ranks changed for many of the genetic entries at the low density planting. This suggests that there is genetic by spacing interaction taking place within many of the genotypes. More genetically homogenous genotypes did not show greater stand-level uniformity for height or diameter at breast height (dbh). Full-sib and open-pollinated genotypes had significantly lower CV's than the three clones, from age 1 to 5. However, the coefficient of variation for all genotypes decreased as age increased, suggesting genetic homogeneity may be more evident in phenotypes as trees mature.

AMERICAN SYCAMORE BREEDING STRATEGIES FOR GROWTH MAXIMZATION AND DISEASE RESISTANCE

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An open-pollinated progeny test of Westvaco's High Wood Density American Sycamore (*Platanus occidentalis* L.) Seed Orchard, selections from their First-Generation Seed Orchard, selections from the 1983-1984 Limited-Range Provenance/Progeny Test, and six control-pollinated families was established in 2002 and 2003. All 55 open-pollinated families were planted at two sites in western Kentucky and south-eastern Missouri. The control-pollinated families were generated from selections that exhibited disease resistance and susceptibility to a variety of diseases, where symptoms included bronzing of leaves and crown dieback. A mating design using resistant and susceptible parents resulted in two families representing resistant by resistant, resistant by susceptible, and susceptible by susceptible. These six full-sibling families were also incorporated with the half-sibling plantings and were given to the US Forest Service Center for Bottomland Hardwood Research for testing near Stoneville, MS. All Stoneville trees were challenged by inoculation fall 2002 with the leaf-scorch-causing bacterium, *Xylella fastidiosa*. Diameter and height data were recorded at ages three, five, seven, and nine. Bacterial leaf scorch disease presence on all Stoneville families was recorded as symptomatic or asymptomatic/mildly symptomatic. Among the half-sibling families at four ages of measurements (i.e., three, five, seven, and nine) the average family heritability was 0.59 for both height and diameter and 0.53 for volume. These strong heritability values indicate that superior growth can be captured through family selection. Furthermore, age-age correlations indicate that making selections based on age-five data results in the greatest precision for gains at age nine for half-sibling families. Analysis of presence and absence of disease among control-pollinated families also indicates that breeding of bacterial leaf scorch resistance can be achieved simply through crossing two parents that show resistance. This results in slightly more than a 4.5 fold decrease in the probability of infection by age nine. Overall, these results indicate that sycamore has a large capacity for improvement through traditional breeding.

THE GENOMES OF GIANTS: A COMPARATIVE WALK THROUGH THE FOREST OF TREE GENOMES

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In the past decade, significant advances in the genetics and genomics of fruit and forest trees have led to the development of the tree genome models. These models are currently being exploited to uncover the genes and gene networks that control the myriad of important traits that define our tree resources. To often however, the significant advances in one species are not rapidly translated to other species due to the lack of our understanding of the structural and functional genomics similarities and differences among species in different families. Comparative genomics analyses provide the avenue to explore the evolution of tree genomes often providing details of genes and gene networks that impact characters important to both the forest and fruit tree sustainability. In this presentation the application of such comparative strategies will be presented in the context of characters that significantly impact the fruit and forest tree industries and examples of the cross species utility of the model genomes for candidate gene discovery will be provided as a roadmap for advancing our understanding of tree genetics.

EVOLUTION, REGULATION AND MANIPULATION OF *POPULUS TUBULINS*: THE USUAL SUSPECTS WITH UNUSUAL CONSEQUENCES

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Cortical microtubules are cytoskeletal components that have important roles in morphogenesis. Of particular relevance to the bioenergy and forest products industry is the postulated role of microtubules in orchestrating cellulose microfibril deposition during cell wall formation. The microtubule component proteins α- (TUA) and β-tubulins (TUB) are encoded by multi-gene families with very high overall sequence homology across species. We have previously characterized the spatiotemporal expression patterns of the *Populus TUA* and *TUB* families (Oakley et al. 2007). In addition to identifying several xylem-abundant and bending-responsive isoforms, we found unusual sequence heterogeneity at the C-termini, the post-translational modification (PTM) hot-spot in animal tubulins. To investigate tubulin function during wood formation, we developed a suite of transgenic *Populus* that exhibit perturbed *TUA* to *TUB* transcript ratios, or that express tubulin PTM mimics. Most of the construct combinations resulted in abnormal organogenesis and vascular development, and failed to produce viable plants. Only three of the combinations led to whole-plant regeneration, and interestingly, all three featured the C-terminal variants. The transgenic trees appeared morphologically normal, but exhibited a range of epinasty and twisting in mature leaves. Bark color was noticeably lighter in the transgenics. Lignin content and lignin structure were differentially altered in the transgenics. The results are consistent with a function of microtubules and microtubule PTMs for plant development and cell wall biogenesis in *Populus*, and offer novel strategies to manipulation of wood properties.

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HYPER-ACCELERATING BREEDING AND ADAPTATION OF LOBLOLLY PINE USING GENOMIC SELECTION

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Increasing in demand for wood products and growing evidence of climate change creates a pressing need for the development of more productive germplasm that is adapted to existing and novel sources of biotic and abiotic stress. Marker-assisted selection (MAS) was previously proposed as an approach to accelerate genetic improvement of conifers. However, the very limited proportion of the total genetic variation captured by the markers identified in association studies hinders their practical application in breeding program. Alternatively, the combined effect of all available markers may be estimated simultaneously, and used to predict the genomic breeding value of progeny in future generations. This approach of Genomic Selection (GS) has become widely adopted in animal breeding and is now of increasing interest to tree breeders. GS greatest impact is expected in breeding of conifers, by significantly reducing the breeding cycle and facilitating early selection of traits expressed late in the rotation, with low heritability. In this presentation the implementation of GS in conifer tree breeding programs will be discussed in light of our experience in developing prediction models for a breeding population of loblolly pine. The results to be discussed demonstrate the feasibility and remarkable gain that can be achieved by incorporating GS in breeding program of conifers, compared to traditional breeding. However, there are clear limitations in the use of prediction models across breeding zones and ages, and obstacles in model transferability across populations should not be underestimated.

TOWARDS A POPLAR BIOMASS PROTEIN-PROTEIN INTERACTOME

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Interactions between proteins are central to nearly all biological functions. We are identifying and mapping *Populus* protein-protein interactions relevant to woody biomass formation by focusing on proteins that are coexpressed in developing secondary xylem. Through integration with other 'omics data, this high-confidence wood interactome will provide a solid framework for identifying key regulators of wood formation and biomass accumulation and for designing strategies to alter biomass traits. We cloned 374 members of the poplar biomass ORFeome for use in indentifying biomass protein-protein interactions. These biomass ORFs encode a variety of protein classes, such as proteins involved in cell-wall synthesis and signal transduction as well as proteins of unknown function. Completion of yeast two-hybrid (Y2H) binary assays involving over 300 biomass ORFeome members has identified 11 interaction pairs. 60 biomass ORFeome members are being used as bait proteins for an Y2H screen with a poplar xylem cDNA prey library. Nine bait proteins have be completely through the screening process with 43 unique high-confidence protein-protein interactions identified. Methods and results from these Y2H screens as well as diagrams of identified biomass protein interaction modules are available at our project website (http://xylome.vbi.vt.edu/index.html). In addition, functional analyses *in planta* of selected interacting proteins can provide valuable insight regarding new strategies for regulating woody biomass production. Thus, we have begun to functionally characterize select interacting pairs in both Arabidopsis and poplar by ectopically expressing or suppressing genes singly and in combination. Co-overexpression of interacting proteins PB15 (ROP-GTPase) and PB129 (DUF620) in Arabidopsis resulted in expanded interfascicular regions containing enlarged fibers compared to fibers in normal interfascicular regions of the inflorescence stem. Notably, this phenotype was not observed in transgenics overexpressing just one of these genes, showing the potential of interactome data to be translated into alteration of wood phenotypes.

CONIFER TRANSLATIONAL GENOMICS NETWORK: BRINGING GENOMICS-BASED BREEDING TO APPLICATION

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The Conifer Translational Genomics Network (CTGN) consists of researchers from six institutions representing tree improvement cooperatives, genomics laboratories, and USDA Forest Service research projects responsible for developing most of the conifers planted in the United States. The goal of the CTGN project is to provide tree breeders with the genomic based tools to make tree breeding both more effective and efficient. The four-year project, funded by the USDA National Institute for Food and Agriculture (formerly CSREES) and the USDA Forest Service, seeks to leverage more than 50 years of population development conducted by the tree improvement cooperatives with the genomic and population genetics skills provided by researchers at those same institutions and UC Davis. Additional activities supported by the project include comprehensive education and outreach programs and the development of a genetic stock center for both southern pine and Douglas fir. While each institution has its own research emphasis, all are based on the use of genotyping large numbers of single nucleotide polymorphisms (SNPs) for substantial numbers of individuals. These data are being used to characterize genetic variation in managed populations, seek signatures of natural and artificial selection, and improve selection efficiency through marker-trait association and development of better analytical tools. Just as the CTGN was built on previous research, one of its chief accomplishments has been to provide impetus and tools for future projects, most notably the recently announced Pine Reference Sequence Project and the Southern Pine Climate Change Mitigation and Adaptation Project. A brief overview of significant progress emerging from the CTGN will be presented.

MODIFYING LIGNIN TO IMPROVE THE UTILITY OF *POPULUS* AS A BIOENERGY CROP

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As a perennial woody plant, hybrid poplars (species within the genus *Populus*) offer several advantages as a bioenergy crop, including rapid growth rates, the ability to cycle nutrients, a wide geographic distribution, genetic diversity, amenability to genetic engineering, abundant genomic resources, greater flexibility with regard to harvest, and more efficient transport and storage. The phenolic cell-wall polymer lignin constitutes a significant barrier to biomass conversion for all cellulosic feedstocks; however, it is also essential to normal plant growth and development. Recent advances in our understanding of how lignin is synthesized provide options for modifying the composition of lignin, in an effort to improve conversion efficiencies. In poplar, we have over-expressed various genes involved in the lignin biosynthetic pathway, and used RNA interference (RNAi) to down-regulate others.

Recently, we have begun testing the maize *Corngrass1* (*Cg1*) gene, which encodes a unique MIR156-class microRNA, in poplar. In herbaceous species, *Cg1* overexpression fixes plant development in the juvenile phase and, thus, affects the initiation of meristems and lateral organs. Plants in which it has been expressed constitutively produced multiple axillary branches, grew faster, contained less lignin, and were either sterile or exhibited delayed flowering. We have over-expressed *Cg1* in poplar and our results are consistent with those seen in other species.

Materials and Methods

Lignin-modification vectors

To alter lignin quantity and quality, we manipulated the expression of four genes in the lignin biosynthetic pathway: *C4H*, *C3'H*, *F5H*, and *COMT*. This combination allows us to evaluate the impact of decreased lignin content (*C4H*, *C3'H*), as well as changes in monomer composition (*C3'H*, *F5H*, *COMT*) on the ease with which cell walls can be deconstructed. Combined *F5H* up- and *COMT* down-regulation should result in plants with 5-hydroxyguaiacyl subunits. This type of lignin is not known to occur naturally, but could be of interest with respect bioenergy. Poplar cDNA clones were provided by Jörg Bohlmann (UBC). *Arabidopsis F5H* cDNA was previously isolated (Meyer et al. 1996). To generate entry clones, fragments were restricted from cDNA clones and sub-cloned into an entry vector. To generate RNAi constructs, entry clones were recombined with a destination vector using the Gateway LR cloning system. To generate over-expression vectors, entry clones were recombined with over-expression destination vector using LR. To drive expression of RNAi transcripts, we used both the CaMV *35S* and the *Arabidopsis C4H* promoters.

Corngrass1 vectors

A 0.6-Kb DNA fragment containing the *Cg1* transcript (Accession # EF541486) was cloned into the binary plasmid pK2GW7 (Karimi et al. 2002) by recombinase-mediated integration using LR clonase, positioning the *Cg1* transcript downstream from the 35S promoter and upstream of the *35S* terminator.

Producing transgenic plants

T-DNA from binary vectors was transformed into hybrid poplar clone INRA 717-1B4 (*Populus tremula* x *P. alba*) using an *Agrobacterium*-mediated transformation protocol (Meilan and Ma 2006). A minimum of 25 lines (independent transgenic events) was produced for each construct. Transformation of kanamycin-resistant rooted lines was verified via PCR; transgenic plants were acclimated in a greenhouse and shade frame before being transplanted outdoors (4 ramets/line). Material from field-grown trees was used to conduct analyses on lignin-modified plants; material from greenhouse-grown plants was used for analyses performed on the *Cg1* transgenics.

Lignin analyses

Lignin composition and content were determined using standard methods (i.e., Klason lignin, pyrolysis GC-MS, and DFRC analysis) that have been described previously (Franke et al. 2002).

Quantitative real-time PCR

Total RNA was extracted from the youngest fully unfurled leaf using the RNeasy Plant Mini Kit (Qiagen). Primers were designed using PrimerExpress, and reverse transcription were performed using the High-capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). Reactions (3 technical replicates) were performed using Fast SYBR Green Master Mix (Applied Biosystems). Expression level was normalized to that of the alpha tubulin gene (*TUA2*).

Plant measurements

Three ramets (biological replicates) of *in vitro* regenerated WT and 35S:*Cg1* transgenic lines, all at 5 months of age, were measured for height, stem diameter (5 cm above soil level), number of branches, number of leaves, number of nodes, internode lengths, and rate of leaf initiation. Plastochron index was measured as described by Erickson and Michelini (1957).

Results and Discussion

Because of position effects, the RNAi vectors were effective to varying degrees in the lines. For each vector, the 8 lines with the lowest level of native gene expression were propagated for transfer to the field. Relative expression for the *COMT* RNAi lines is shown in Figure 1. Syringyl (S) content of lignin in WT poplar is ~60%; our best-performing *COMT* RNAi lines had an S content of <10%. Currently, we are using plant material from field-grown trees for pre-

treatment and hydrolytic experiments. In addition, we are collaborating with an entomologist and pathologist to evaluate the transgenics' susceptibility to pests.

When *Cg1* was over-expressed in poplar, plants exhibited significantly greater branching and leaf area, larger stipules, and shorter internodes, but the number of internodes remained unchanged (Figure 2). The increased leaf area was due to sylleptic branching, but the rate of leaf initiation was unaffected. The severity of the phenotype was positively correlated with *Cg1* expression level. In addition, transgenic lines had up to 30% less lignin and syringyl to guaiacyl ratio (S/G) was lower than in WT poplar or a control transgenic line having low *Cg1* expression. We are in the process of establishing a field study to compare biomass production of our *Cg1* and WT lines. It is yet to be determined whether MIR156 directly regulates lignin biosynthesis or if the observed lignin changes were indirect consequences of the developmental changes caused by *Cg1* over-expression. Nevertheless, plants expressing *Cg1* may have commercial value as a cellulosic feedstock for biofuel production and in the paper-manufacturing industry.

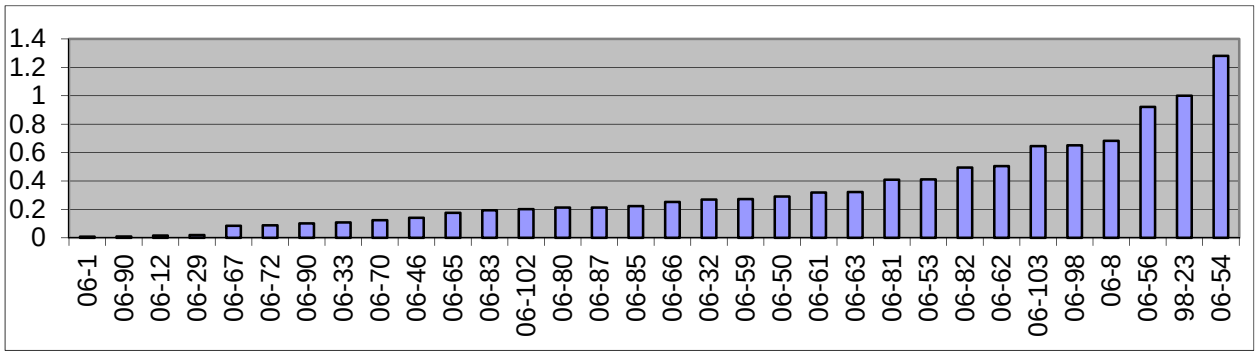

Figure 1. Relative expression of *COMT* (Y axis) for transgenic poplar lines (X axis) in which *COMT* was down-regulated using RNAi.

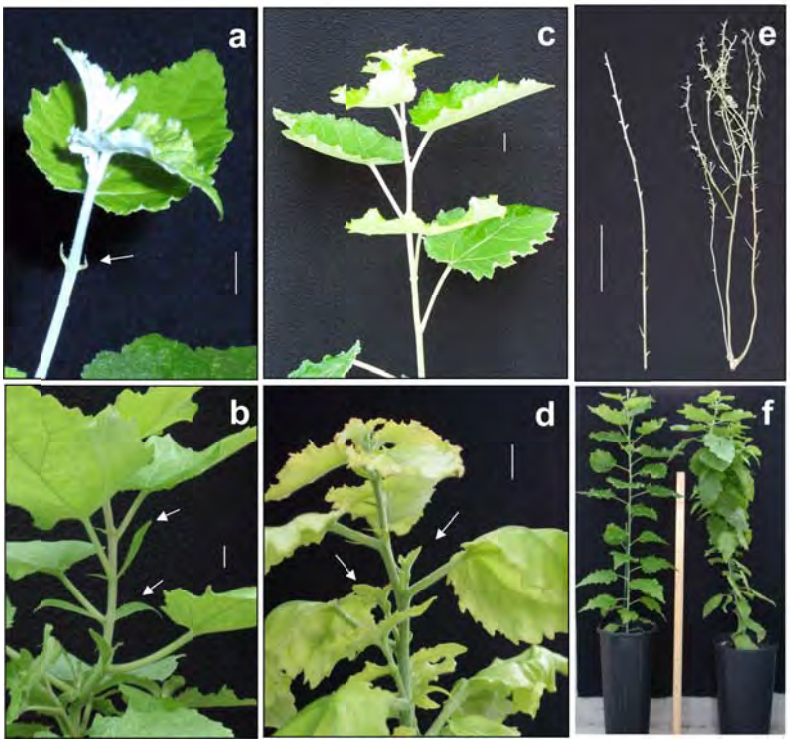

Figure 2. Comparison of wild-type (WT) and 35S:Cg1 poplar (INRA 717-1B4). A) Shoot apex of 5-month-old WT with stipule (arrow). B) Shoot apex of 5-month-old 35S:Cg1 with enlarged stipules (arrows). C) Shoot apex of 5-month-old WT. D) Shoot apex of 5-month-old 35S:Cg1 plant showing outgrowth of sylleptic branches (arrows). Scale bars in panels A, B, C, and D = 1 cm. E) An ~40-cm apical shoot from 1-year-old WT (left-hand side) and 35S:Cg1 (right-hand side) plants. Leaves were removed and petioles shortened to show branching. Scale bar = 10 cm. F) 7-month-old whole plants (WT on the left, 35S:Cg1 on the right). Ruler = 1 m.

Acknowledgements

This work was funded by grants from the U.S. Department of Energy, Office of Science (DE-FG36-04G01417), and the Energy Center, Purdue University.

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CHASING OPPORTUNITIES OR IS IT THE OTHER WAY AROUND?

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Tree improvement research in the South has transformed the production and processing of wood for traditional and non-traditional applications. While it has solidified South's role as the wood basket of the United States, significant challenges still lie ahead in meeting the ever increasing demand for wood and woody biomass. The emerging bio-based economy where woody biomass could contribute significantly to produce advanced biofuels and bio-based products has opened up new opportunities and challenges. Development of such bioenergy systems provide expanded markets and ecosystem services such as carbon sequestration and biodiversity conservation. However, concerns about their water and nutrient use patterns and impacts on water quality and quantity have surfaced over the years. While acknowledging the advancements made, this talk will challenge the tree improvement community to respond rapidly to emerging issues and opportunities.

INTRODUCTION AND PROVENANCE TRIALS OF EASTERN WHITE PINE (*PINUS STROBUS*) IN KOREA

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Introduction of eastern white pine (*Pinus strobus*) was started in early 1900's with small scale in Korea. The real provenance trial sets were established in 1964 with five provenances at one site. Three experimental sets for provenance trials were analyzed in this study. *P. strobus* were used to analyze the growth performance of provenances over sites, growth patterns, provenance by site interaction and correlation between ages. The superior provenance of *P. strobus*, suitable environmental conditions were proved.

Materials and Methods

Set 1: As a first provenance trial of *P. strobus*, five provenance seeds (four provenances from north America and one from Canada) had been introduced and established at one site (Hwasong city) in 1964. Growth performance was analyzed among provenances at age 20.

Set 2: Second provenance test set were established with two provenances and four seed sources (USA, Italy, and New Zealand) over four sites in 1972. The growth and growth pattern over ages were studied among provenances. The growth of *P. strobus* was used to analyze provenance by site interaction, correlation between growth and environmental conditions and between ages.

Set 3 : Third set was established with six provenances (from USA) at one site (Hwasong city) in 1986. Growth performance and growth pattern over ages were analyzed.

Results and Discussion

Set 1: The growth performance of *P. strobus* by provenances was investigated over ages. The height (5.0 m) and D.B.H. (9.4 cm) of New York provenance was best at age 20. The growth of Pennsylvania (height 4.3 m, D.B.H. 7.2 cm) was worst. The volume of NY provenance was over 3 times than reference pine (*P. koraiensis*). The needle length of NY provenance was 10.3 cm, and that of Ontario provenance was 7.1 cm.

Set 2: Growth and growth pattern of provenances varied over sites. The volume growth at age 39 was best at the Chuncheon site among the four sites, and that of North Carolina provenance was proved to be superior in every site. Growth pattern of height and diameter were very different between provenances and sites. Height and diameter growth were positively correlated with ages. Height growth was positively correlated with annual precipitation, number of foggy days and sand contents in the soil while diameter growth was positively correlated with longitude, altitude and clay contents in the soil of the test sites. Variance component analysis revealed that there is a provenance by site interaction in diameter growth but no interaction in height growth. The

portion of interaction tern of total variation explained 2.0~2.5% in height and 18.9~24.6% in diameter of the total variation according to the analysis of covariance and AMMI model, respectively. North Carolina provenance was proved to be best provenance with good adaptability (stability) and performance and New York provenance was worst.

Table 1. The mean individual volume growth of the *P. strobus* provenances and seed sources at age 39 at the four test sites in Korea (m^3)

	Chun-cheon	Gunpo	Cheong-ju	Imsil	mean
New York	0.832^{b}	0.489^{a}	0.451^{c}	0.515^{b}	0.599^{b*}
North Carolina	0.936^{a}	0.495^{a}	0.612^{a}	0.827^{a}	0.727^{a}
Rotorua	0.666^{c}	0.306^{c}	0.586^{ab}	0.515^{b}	0.605^{b}
Induno Olona	0.855^{ab}	0.409^{abc}	0.551^{ab}	0.717^{a}	0.626^{b}
Bagnolo	0.648^{c}	0.381^{bc}	0.548^{b}	0.502^{b}	0.547^{c}
Ternavasso	0.807^{b}	0.441^{ab}	0.535^{bc}	0.676^{ab}	0.615^{b}
P. koraiensis	0.390	0.110	0.482	0.262	0.324

Table 2. Comparison of the interaction portion of the total variance from the covariance analysis and AMMI analysis for height and DBH of *P. strobus* provenances at age 20 and 39

Age	20		39	
	Analysis of covariance	AMMI	Analysis of covariance	AMMI
Height	1.2	1.0	2.5	2.0
DBH	6.4	5.2	24.6	18.9

Set 3: The results of the 27-year-old *P. strobus* provenance test with six provenances indicated that the growth of the southern provenances (Georgia, North Carolina) were superior to the northern provenances (Minnesota, Wisconsin). At age 27, the annual height growth was still increasing while diameter growth was gradually decreasing.

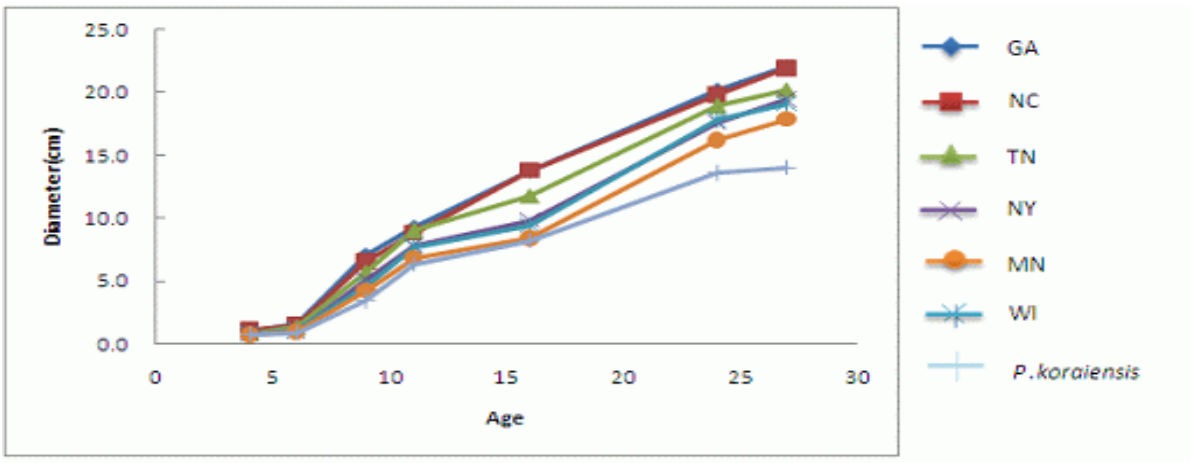

Figure 1. DBH growth pattern of *P. strobus* provenances over ages at Hwasong test site in Korea

Table 3. The Pearson's correlation coefficient of DBH growth of the provenances of the '86 provenance test among ages in Hwaseong

	4	6	9	11	16	24	27
4							
6	0.872*						
9	0.851*	0.975**					
11	0.783*	0.949**	0.957**				
16	0.934**	0.989**	0.969**	0.934**			
24	0.747ns	0.909**	0.950**	0.957**	0.892**		
27	0.740ns	0.881**	0.941**	0.922**	0.869**	0.992**	

Conclusions

In case of *P. strobus*, the growth of southern provenance was better than that of northern provenance in general. The growth was positively correlated between ages, it means that early selection of superior provenance would be possible. The result of provenance test, provenance by site interaction and growth pattern revealed that North Carolina was the best provenance in Korean environments.

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HYDROTHERMAL LIQUEFACTION OF PERENNIAL BIOMASS FEEDSTOCKS FOR PRODUCTION OF TRANSPORTATION FUELS

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Biomass as renewable energy sources is receiving worldwide attention for several reasons. These include the desire to develop sustainable energy sources, decreasing dependence on oil, and decreasing the rate of depletion of the fossil fuel reserves. Perennial grasses had many characteristics make them an ideal alternative energy sources. They can potentially be produced in reliable quantities with greater inexpensive price stability, also, they can provide extensive environmental benefits to soil, water and air through reduction of hazardous gas emissions. The aim of this work is to utilize some perennial grasses greatly available at Southern United States such as switchgrass and giant miscanthus for production of transportation fuels. The promising hydrothermal liquefaction (HTL) conversion processes will be applied for this study. The most important parameters that effect hydrothermal liquefaction as time, pressure, temperature and catalyst will be studied. Full physical and chemical characterization for the properties of the produced bio-oil will be performed. Finally, the bio-oil with the best physical and chemical properties will be upgraded into liquid bio-fuels and some evaluation tests will be performed.

INFLUENCE OF AMERICAN VERSUS CHINESE CHESTNUT GENOME PROPORTION ON SOMATIC EMBRYOGENESIS INDUCTION

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Somatic embryogenesis (SE) has been successfully applied for clonal propagation of American chestnut trees, including transgenic trees engineered with genes potentially conferring resistance to chestnut blight. It may also be useful for clonally propagating blight-resistant trees produced by a hybrid backcross breeding program, in which American trees are hybridized with blight-resistant Chinese chestnut trees, followed by backcrossing to American trees. However, to date there have been no reports of SE in either Chinese chestnut or hybrid backcross material. We tested the effects that Chinese chestnut genome proportion and pollination type (control versus open pollination) have on the success of SE induction using a standard protocol for culturing American chestnut. None of the material that had been control-pollinated produced embryogenic cultures. Hybrids, which had 50% Chinese chestnut genome, were also unsuccessful. Open-pollinated American chestnut and open-pollinated hybrid backcross B3F3 trees (approximately 15/16 American and 1/16 Chinese) successfully produced embryogenic tissue from the SE induction. The first B3F3 somatic embryos are now in production, offering the potential to clonally propagate elite advanced generation hybrid backcross trees for restoration, as well as for timber and nut production.

REGULATION OF REPRODUCTIVE ONSET AND VEGETATIVE GROWTH IN POPLAR

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Unlike annual plants, trees show repeated cycles of transition between vegetative and reproductive growth at sexual maturity. However, it is not clear how trees coordinate vegetative and reproductive growth without a developmental conflict. Through manipulative physiological and genetic experiments coupled with field studies, expression profiling, and network analysis, we show that the whole genome duplication products *FLOWERING LOCUS T1* (*FT1*) and *FLOWERING LOCUS T2* (*FT2*) coordinate these two important processes in poplar (*Populus* spp.). We will present our experimental findings in detail and discuss how they could provide new insights into tree improvement via breeding and biotechnology.

GENETIC VARIATION AND STRUCTURE OF NATURAL POPULATIONS IN *ABIES HOLOPYLLA* MAXIM. EPLOYING ISSR MARKERS

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Introduction

Fir (*Abies*) genus in Korea has Needle Fir (*Abies holopylla* Maxim.), Korea Fir(*Abies koreana* E.H. Wilson) and East Siberian Fir [*Abies nephrolepis* (Trautv.) Maxim.]. Needle Fir widely distributed in the mountains such as Mt. Seorak, Mt. Odae and Mt. Taebaek. Indigenous species are distributed on altitude of 200 ~ 1,400 meters above sea level. The purpose of this study was to provide the genetic data for *in-situ* and *ex-situ* conservation.

Materials and Methods

Young leaf samples were collected from 20 individual samples of six natural populations, *Abies holopylla*, in South Korea. The selection of individual samples was made in such way that they are apart at least 30 meters away from other individule sample in order not to select a related tree. After screening for total 46 UBC primers, 6 primers were analyzed to estimate the genetic variation, genetic structure and relationships based on observed allele by PCR analysis.
To estimate the distribution of I-SSR variants among the categories of presence or absence within population, the Shannon's index (Shannon 1948) was calculated using the POP-GENE 1.31 program (Yeh et al. 1999). Level of genetic differentiation among populations was estimated by AMOVA on the basis of genetic distance using Arlequin 2.0 program (Scheider et al. 2000). Genetic relationships among populations were reconstructed by UPGMA on the basis of pair-wise Manhattan distance (Wright 1978) between populations, which was computed by RAPDDIST v. 1.0. Statistical test for the topology of each node was performed with 100 bootstrapped samples prepared by RAPDDIST v. 1.0 (Wright 1978)

Results and Discussion

From these results, we found that relationship among populations by analyzing genetic variation and genetic structure of six populations using ISSR (Inter Simple Sequence Repeat) primers. Genetic diversity was the highest in population of Mt. Odae (*S.I* = 0.469), while population of Mt. Heungjeong (*S.I.* = 0.403) was the lowest (Table 1). These degrees of genetic diversity were higher than other deciduous trees such as *Oplopanax elatus*, its degree of genetic diversity was 0.187 (Lee et al., 2002) in Korea and *Kirengeshoma palmate*, its degree of genetic diversity was 0.259 (Zhang et. al. 2006) in China. This is because endangered plant species have low genetic diversity due to the genetic drift and gene flow (Karron 1991).

Table 1. Genetic diversity in 6 populations of of *A. holopylla* in South Korea.

Population	N	*S.I.*[*]
Mt. Seorak	20	0.419
Mt. Odea	20	0.469
Mt. Heungjeong	20	0.403
Mt. Undal	20	0.405
Mt. Unmoon	20	0.433
Mt. Jiri	20	0.445
Mean	**20**	**0.429**

[*] *S.I.*: Shannon's information index

Genetic diversity of an average 0.429 of the species level showed similar level, when compared with the studied species up to now and the others species similar to ecologic and life historic characteristics. The results by AMOVA (Analysis of Molecular Variance) on six populations of *A. holopylla* showed that 5.61% of total genetic variation was caused by the difference among populations and 94.39% of the others were caused by between the individuals within populations.

It is more effective and economical to conserve species by selecting many individuals within a population rather than selecting many populations for *ex-situ* conservation. You also can conserve species intensively by selecting a few populations rather than selecting many populations for *in-situ* conservation.

Table 2. Analysis of molecular variance within/among populations.

Source of variation	d.f.	Percentage of variation (%)
Among populations	5	5.61
Within populations	114	94.39
Total	**120**	**100**

From the UPGMA cluster analysis results, geographically close groups tended to be grouped into the same group (Figure 1), and showed positive correlation ($r = 0.827$, $p < 0.01$) between geographic distance and genetic distance for populations (Figure 2).

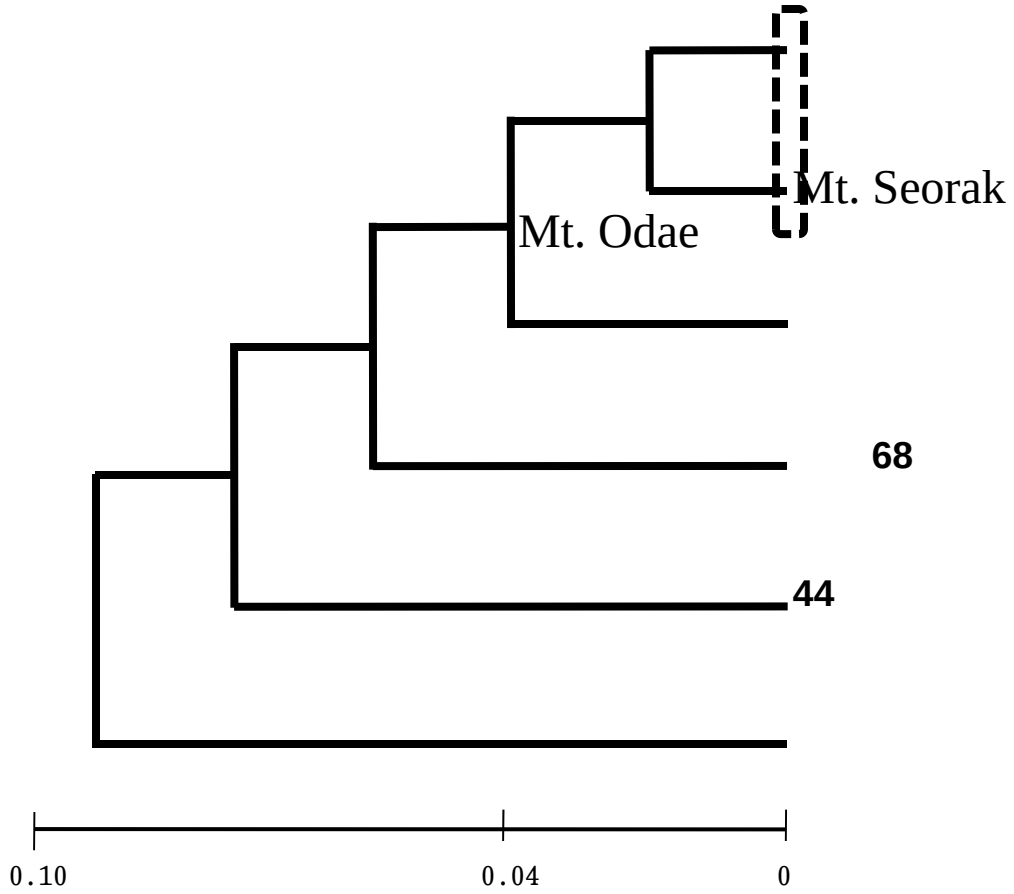

Figure 1. UPGMA dendrogram of *A. holopylla* populations.

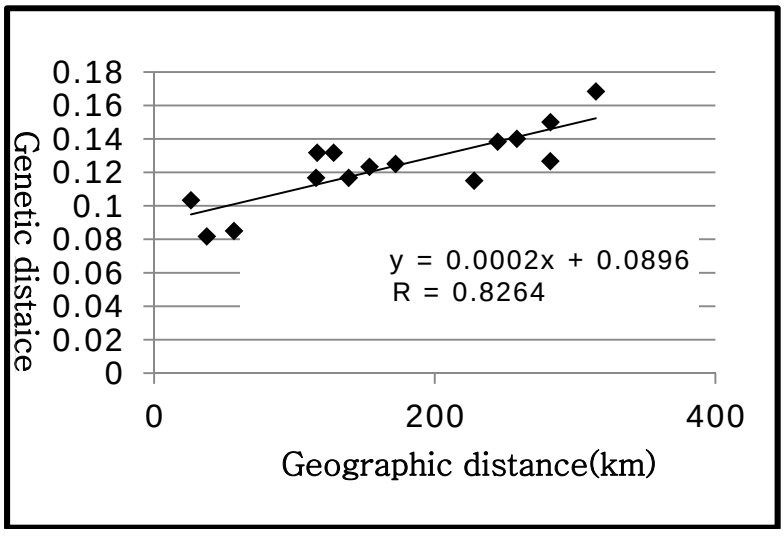

Figure 2. The correlation between genetic distance and the geographic distance for *A. holopylla*.

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MASS PRODUCTION OF A RARE AND ENDANGERED SPECIES, *ASTRAGALUS MEMBRANACEUS* VAR. *ALPINUS* NAKAI, THROUGH AXILLARY BUD CULTURE AND IN VIVO ROOTING TEST

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Introduction

Astragalus membranaceus var.*alpinus* Nakai is a native plant to Jeju Islands (South Korea). This rare species is growing at 1,600 m above sea level in Halla mountain. In Jeju Islands, it is assumed that the number of native communities is 5~10. This plant has high scientific value and it was designated as a critically endangered by IUCN. This study was conducted to search the suitable propagation conditions by axillary bud culture and to identify the rooting characteristics of *A. membranaceu*s via rooting test.

Materials and Methods

Plant materials

Explants of *A. membranaceus* were provided from Mt. Halla located at Jeju Islands in Korea. The plantlets were firstly cultured on MS medium. After first culturing, 7-week axillary buds were sub cultured and tip stems were maintained to produce shoots and roots. The length of explants with all leaves was 2cm in *in-vitro* culture test and 3.5cm in *in-vivo* rooting test, respectively. All tests were done in laboratory conditioned at 25±1 °C 16hr fluorescent light (40μmol/m^2/s) and 8hr dark.

Axillary bud culture

Nodal segments for selecting suitable medium were incubated in two kind of medium, MS and WPM, to which various kinds of cytokinin (Kinetin, BAP, Zeatin; each 0.2, 0.5, 1.0 mg.L^{-1}) had been added. Fifty explants were used per treatment. After 4 weeks, the length of explants, number of new shoot, total leaves and callus induction were measured.

Rooting test

In-vitro rooting test

Media screening for *in-vitro* rooting tests was firstly done and WPM medium was selected. On this medium, auxin hormones such as NAA, IAA, IBA, 2.4-D were added to investigate the optimal culture condition. The concentration of these hormones was 0.1, 0.3, 0.5, 1.0, 3.0 mg.L^{-1} and total of 20 explants were plated. After 6 weeks, the number of roots, root length and root status were measured.

In-vivo rooting test

In *in-vivo* tests, hormones of NAA, IAA, IBA were used with concentrations of 50, 100, 300, 500mg$.L^{-1}$. Shoots of 3.5cm were planted in plastic pots (length 45cm * width 25cm * height 10cm) covered with transparent acrylic board and filled with instant bed soil. After 6 weeks, the number of roots, root length and root ratio were investigated from 20 explants. The humidity was 90~95% in first 3 days and after that irrigation was done once in every 3~4 days.

Acclimatization

Rooted plants were replanted in pots filled with instant bed soil and transferred under shading nursery. 4 weeks after, growth status of all tested plants was observed

Results and Discussion

Axillary bud culture

After 4 weeks of culture, Effective basic medium was MS medium for propagation of *A. membranaceus*. In MS medium, the length of explants was longer 2 times than in WPM medium. Nodal segments cultured on MS medium with different cytokinins and the results were shown in the (Table.1).

Table 1. Effect of plant hormones on *in-vivo* propagation in *A. membranaceus*.

Plant hormones ($mg.L^{-1}$)		Length of explant(cm)	No. of shoot(EA)	No. of leaves (EA)	Callus induction
Cont.		2.1	-	10.7	x
Zeatin	0.2	4.1	1.2	10.5	O
	0.5	6.7	0.8	23.9	
	1.0	5.6	0.4	22.7	
BAP	0.2	5.0	0.4	32.6	O
	0.5	4.5	2.0	23.9	
	1.0	5.1	1.8	22.9	
Kinetin	0.2	5.8	0.2	18.1	O
	0.5	5.9	0.8	17.0	
	1.0	5.4	0.6	22.1	

Rooting test

In-vitro rooting test

The rooting rate was doubled high in NAA 0.1 mg.L^{-1} as 40% comparing to hormone non treated (Table 2). The numbers of roots were relatively good in NAA treatments and the root length was best in the treatments of IBA 1.0~3.0 mg.L^{-1} (Fig 1).

Table 2. *In-vitro* rooting rate of *A. membranaceus* (WPM Medium).

Cont.	NAA (mg.L^{-1})					IAA (mg.L^{-1})					IBA (mg.L^{-1})					2,4-D (mg.L^{-1})
	0.1	0.3	0.5	1.0	3.0	0.1	0.3	0.5	1.0	3.0	0.1	0.3	0.5	1.0	3.0	0.1 ~ 3.0
20%	40 %	20 %	25 %	20 %	25 %	10 %	10 %	5%	-	-	10 %	10 %	20 %	20 %	-	-

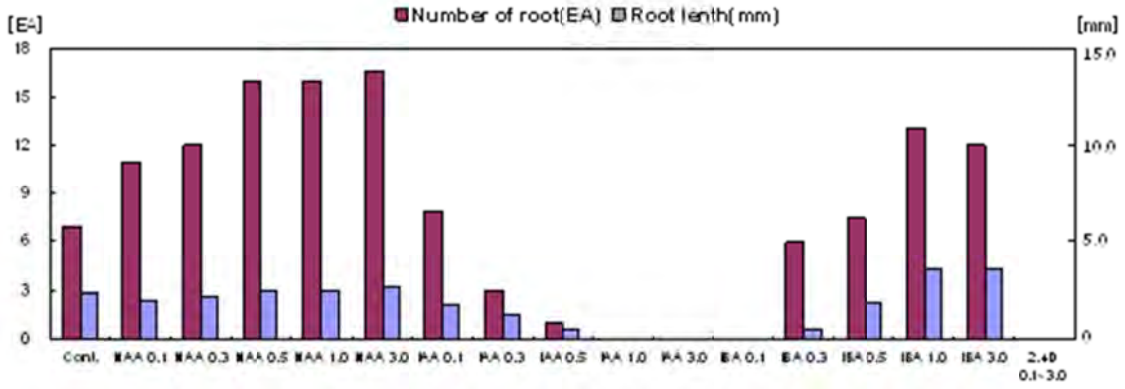

Figure 1. Rooting characteristics of *A. membranaceus* (*in vitro*).

In-vivo rooting test

The rooting rate was doubled high in IBA 500 mg. L^{-1} as 40% comparing to hormone non-treated (Table 3). The numbers of roots were relatively good in NAA treatments and the root length was best in the treatments of IBA (Fig 2, Fig 3(Left)).

Table 3. *In-vivo* rooting rate of *A. membranaceus*.

Cont.	NAA (mg.L^{-1})				IAA (mg.L^{-1})				IBA (mg.L^{-1})			
	50	100	300	500	50	100	300	500	50	100	300	500
30%	60%	45%	50%	30%	25%	45%	35%	45%	60%	55%	55%	70%

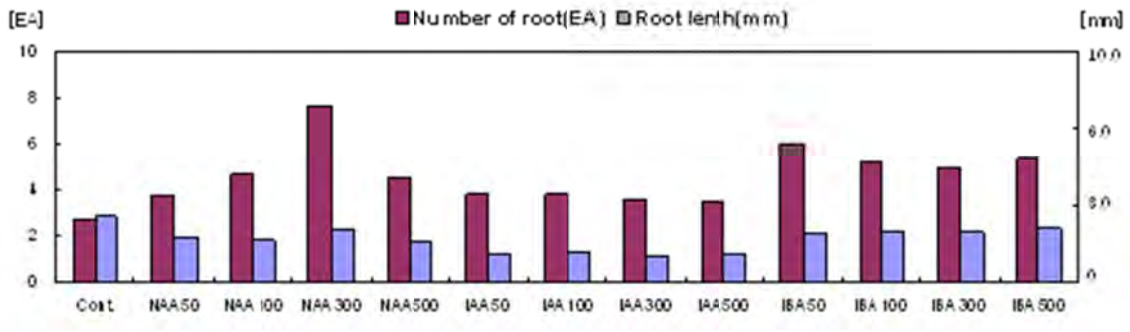

Figure 2. Rooting characteristics of *A. membranaceus* (*in-vivo*).

Acclimatization

In-vivo rooted plants were more vigorous than in-vitro ones. After 6 days of acclimatization, they seemed to shown as naturally grown with good growth form and 100% survival rate. In the next spring, new shoots were emerged from all survived plants. The average height was 20cm and it grew to 45 cm 6 month later (Fig 3(right), Fig 4).

Figure 3. Left: *In-vitro* rooted *A. membranaceus*, Right: *In-vivo* rooted *A. membranaceus*.

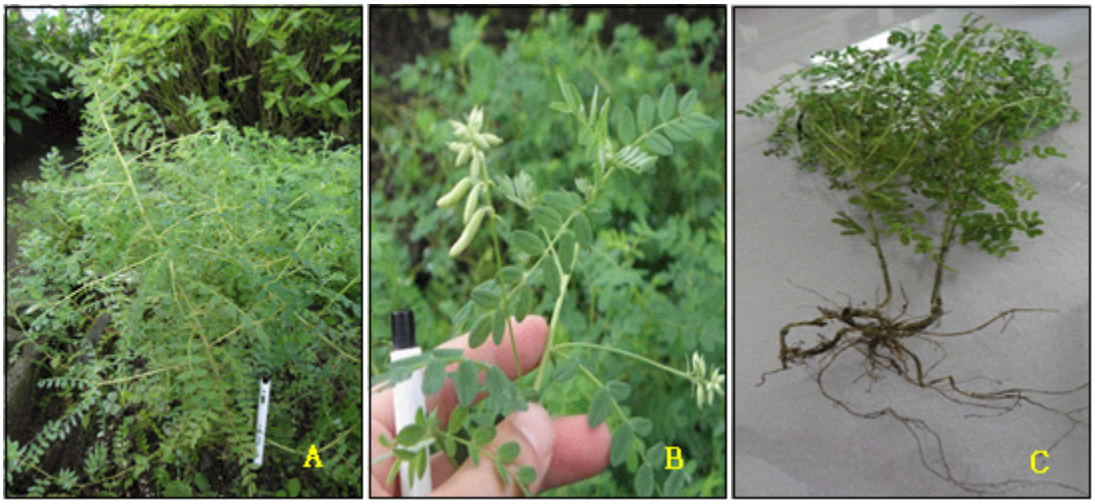

Figure 4. Vigorous grown of *A. membranaceus* in via *in vivo* rooting (A: flower bud, B: root) and naturally grown of *A. membranaceus* on nursery (C).

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GROWTH PERFORMANCE AND ADAPTABILITY OF *LIRIODENDRON TULIPIFERA* IN KOREA

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Generally, exotic species are used when the local indigenous forests cannot or do not produce the desire quantity and quality of forest products. Many regions such as Australia, New Zealand, India, Indonesia and the Middle East, use exotics much of time (Zobel and Talbert 1984). Breeding program for exotic tree species was started in 1924 in Korea. By 1945, total 370 tree species were introduced from 30 countries and tested. However, the plantation and test data were disappeared during Korean War. From 1958 to 1995, total 415 exotic tree species were re-introduced from 38 countries such regions as North America, Europe, Oceania and Asia. Yellow poplar (*Liriodendron tulipifera*) is one of them introduced into Korea at that period. Yellow poplar is naturally distributed most of the eastern USA and is an extremely versatile wood with a multitude of uses such as lumber for unexposed furniture parts and core stock, rotary-cut veneer for use as cross bands in construction of furniture pars, in plywood for backs and interior parts, and as pulpwood (Burns and Honkala 1990). Since 1970's, the growth performance and adaptability of yellow poplar has been tested in Korea. Here, we present the test results and would like to discuss the use of yellow poplar as a reforestation tree species in Korea.

Materials and Methods

To examine the growth performance and adaptability, yellow poplar was introduced from eastern USA in late 1960's and planted at six locations in 1970~1973 (Table 1). Each test stand was classified into three sites such as a good, moderate and poor depending on site index. At each site, three plots (20m x 20m) were set up and the all individuals within the plot were investigated. To compare the growth performance among test plantations, the volume growth of each plantation was standardized to the 28-year old data. The formula is: $E_{VG} = VG/Y \times 28$ where E_{VG} is estimated volume growth at age 28, VG is volume growth of each plantation and Y is the age of plantation. The growth data of *Larix kaempferi* and *Pinus strobus* at Chuncheon (II) and Wanju plantation was also investigated and standardized same method to compare the growth performance among tree species.

Table 1. Details of six test plantations of *L. tulipifera*.

Location	Planted year	Planting space (m)	Area (ha)	Aspect
Anyang, Gyeonggi	1971	4.0 x 4.0	0.5	W
Kwangneung, Gyeonggi	1973	1.8 x 1.8	1.0	E
Imsil, Cheongbuk	1970	1.8 x 1.8	1.0	NW
Wanju, Cheongbuk	1973	1.8 x 1.8	2.0	N
Chuncheon (I), Gangwon	1970	4.0 x 4.0	0.5	N
Chuncheon (II), Gangwon	1973	1.8 x 1.8	2.0	NW

Results and Discussion

The average volume growth per ha for all six plantations was 321m^3, which was similar to that of original habitat in the USA. This suggested that yellow poplar is well adapted to Korean environment. The growth performance of Wanju plantation showed the highest volume growth per ha among the six plantations. It was 4.8 times greater than that of Kwangneung, which showed the lowest volume growth. This difference was due mainly to better atmosphere humidity, soil humidity and depth and clay content. The growth of yellow poplar was nearly 2 times higher than that of *Larix kaempferi* and *Pinus strobus* at Chuncheon and Wanju, which were located even at 350~400m above sea level with steep slope and high ridge. The stem analysis of yellow poplar showed slow growth until the age of 15~20, but grew rapidly after then.

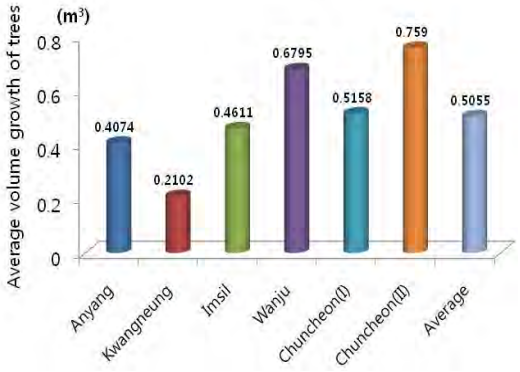

Figure 3. Volume growth of yellow poplar tree species at six different sites.

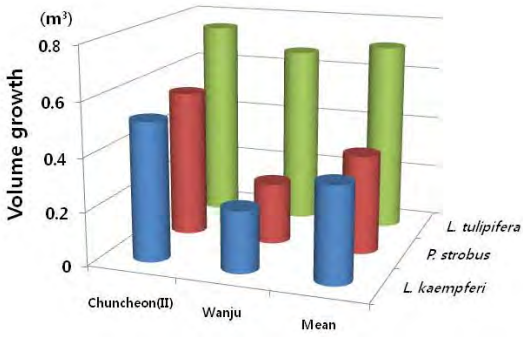

Figure 4. Volume growth of different in two plantations.

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PROVENANCE BY SITE IINTERACTION OF *QUERCUS ACUTISSIMA* IN KOREA

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Quercus acutissima is a native tree species in eastern Asia, in China, Korea and Japan. In Korea, it is a major hardwood tree species in temperate regions and naturally distributed from Hamgyung-do (39°50`) in North Korea to Jeju province (33°20`) in South Korea. Its vertical range is 10~1,100m above sea level. The timber of *Quercus acutissima* has been used for structure wood, tool handle, mine timber, furniture, charcoal and culture medium for mushroom (Cho 1989). Because of its high wood desirability and economic value, the timber consumption and utilization are expected to continue and even increase in the future. Breeding program of *Quercus acutissima* was started with the selection of 207 plus-tree from natural stands in 1990's. Since 1992, total 13.6ha of seedling seed orchards were established. However, the amount of seed production from the seed orchards are not enough to provide seed demand for reforestation due to the differences of flowering and pollination time among individuals in the seed orchards. Thus, large amounts of seeds for reforestation are still supplied from seed production plantations at each province. However, the geographic variation and the growth characteristics of the provenances of *Q. acutissima* are not fully understood. This study was conducted to investigate provenance by site interaction of *Q. acutissima*.

Materials and Methods

To examine provenance by site interaction and stability of *Q. acutissima* provenances, data were collected from three provenance trials established by Korea Forest Research Institute in 1996 with 17 provenances (Tables 1 and 2). The seed sources were systematically selected to cover whole geographic range of *Q. acutissima*. The field trials were established with a randomized complete block design with 3 replications. Each provenance was planted in 10-tree row plot in each block and at a spacing of 1.8m x 1.8m. The data of survival rate and height growth were obtained from measurement at age 12. Data set was analyzed with a linear regression model (Finlay and Wilkinson 1963) to evaluate adaptability and stability of *Q. acutissima* provenances at different environments. The linear regression model is:

$$Y_{ij} = \mu + g_i + E_j + b_i E_j + \bar{e}_{ijk}$$

where Y_{ij} = mean of the *i*th variety in the *j*th environment; μ = general mean; g_i = mean of *i*th variety over all environment; E_j = environmental index for *j*th environment ($Y_{.j} - Y..$); b = regression coefficient; and $\bar{e}_{ijk}$ = residual variation which is assumed to be zero for the values averaged over replications.

Results and Discussion

According to linear regression model, provenance by site interaction effect was significant for height growth ($p<0.001$). The interaction term explained 7.2% of total variation. Most variation

was attributed to environment effect (89.3%). Among the variance of GxE interaction, 15.9% was explained by regression analysis which was based on regression of provenance performance on environment index. The residual GxE interaction was attributed to random deviations. Most of provenances were significantly different from the unity (b=1.0). Adaptability of provenances to test sites were estimated with mean height growth and regression slope (Table 3). Hwasoon, Yeongam and Yeongi provenances are sensitive to environmental change and well adapted to preferable environment. Gangwha, Heungseong, Whaseong, Namyang, Keumreung, Cheongyang and Wonju are less sensitive to environmental change. Particularly, Keumreung showed higher adaptability to poor environment. According to these results, it is suggested that an appropriate provenance to planting site is required for *Q. acutissima*. However, early growth assessments may not be reliable for assessing GxE at mature ages in forest trees (Gwaze et al. 2001; Yeiser et al. 2001). Therefore, long term monitoring of growth performance is required, until mature age. In addition, studies on the relationship between adaptive traits and environments are required to delineate seed zone and achieve advanced breeding for *Q. acutissima*.

Table 1. The location of 17 provenances of *Quercus acutissima*.

Provenances	Latitude(N)	Longitude(E)	Altitude(m)
Gangwha	37°38'	126°28'	15
Heongseong	37°27'	127°59'	218
Wonju	37°26'	128°00'	280
Hwaseong	37°12'	126°59'	50
Namyang	37°11'	126°48'	60
Joongwon	37°04'	127°57'	140
Eumseong	36°52'	127°39'	180
Geosan	36°50'	127°53'	155
Yeongi	36°31'	127°16'	38
Cheongyang	36°26'	126°54'	415
Buyeo	36°21'	126°46'	500
Okcheon	36°19'	127°36'	83

Keumreung	36°14'	128°26'	190
Chilgok	36°04'	128°36'	350
Hwasoon	35°04'	127°03'	540
Boseong	34°49'	127°12'	160
Yeongam	34°49'	126°42'	50

Table 2. The location of three test sites of *Q. acutissima* provenance trials.

Test site	Latitude (N)	Longitude (E)	Altitude (m)
Whaseong, Geonggi province	37°17'	126°56'	100
Chungju, Chungbuk province	36°53'	127°57'	160
Jinju, Geongnam province	35°08'	128°18'	70

Table 3. Stability parameters of *Q. acutissima* provenances.

Provenance	Survival rate				Height growth			
	Mean	b*i*	r^2	S^2d	Mean	b*i*	r^2	S^2d
Gangwha	78.1	0.55	0.86	7.5	331.5	0.69	0.82	25.9
Heongseong	70.0	1.62	0.94	109.4	331.8	0.95	0.87	5.1
Wonju	69.3	0.73	0.57	6.5	309.5	0.81	0.77	3.8
Hwaseong	62.3	1.11	0.89	5.4	337.9	0.84	0.90	6.2
Namyang	76.0	0.98	0.74	41.0	640.5	0.73	0.87	4.4
Eumseong	68.0	0.84	0.86	8.0	313.1	1.69	0.91	145.1
Joongwon	74.7	1.13	0.96	12.3	310.2	1.18	0.86	17.8

Geosan	77.8	0.64	0.97	5.3	321.2	1.36	0.88	55.4
Yeongi	71.7	1.23	0.95	17.4	337.8	1.06	0.76	1.0
Cheongyang	60.3	0.99	0.86	5.4	292.8	0.85	0.85	2.0
Buyeo	77.0	0.86	0.90	5.7	309.2	1.07	0.85	9.1
Okcheon	82.0	0.84	0.86	8.0	320.7	1.08	0.96	5.0
Keumreung	73.0	1.13	0.96	12.3	313.4	0.84	0.89	4.5
Chilgok	62.7	0.64	0.97	5.3	302.7	1.06	0.93	6.6
Hwasoon	79.7	1.01	0.96	6.3	357.7	1.27	0.86	25.4
Boseong	77.7	1.16	0.99	3.6	319.2	1.18	0.91	68.8
Yeongam	72.3	0.79	0.50	12.8	343.8	1.02	0.89	16.6

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APPLICATION OF AIRLIFT BIOREACTORS FOR HIGHLY EFFICIENT GENETIC TRANSFORMATION OF AMERICAN CHESTNUT

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Airlift bioreactors were constructed and applied for proliferation of chestnut embryogenic tissue. More than ten genotypes of American chestnut (*Castanea dentata*) and backcross hybrids of American chestnut and Chinese chestnut (*C. dentata x C. mollissima*) were cultured in bioreactors, of which eight have been used for *Agrobacterium*-mediated genetic transformation. The basal culture medium for bioreactors was woody plant medium (WPM). Medium for tissue proliferation was WPM supplemented with 3 g/l sucrose, 0.5 g/l glutamine and 2 mg/l 2, 4-D. Medium for embryo maturation was the proliferation medium minus 2, 4-D and gelled with 5 g/l Phytagel. In most genotypes, the optimum culture conditions for a one-liter bioreactor included 2% (w/v) tissue density for initial inoculation, 200 ml/min airflow rate, weekly fresh medium feeding (85% fresh medium/15% spent medium, v/v) and monthly fractionation through nested sieves of 1 mm pore size to remove large, old cell clumps. Compared with flasks, bioreactors generated higher yields of tissue mass and larger fractions of tissue consisting of small cell clumps (< 1 mm in diameter) that were suitable targets for transformation. Bioreactor-generated tissue demonstrated high mature embryo yields and high amenability to transformation via *Agrobacterium* co-cultivation. Using bioreactor-grown embryogenic chestnut target material, two reporter genes (*GUSi*, and *YFPGUSi*) and ten candidate genes (CGs) for chestnut blight resistance have been transformed into chestnut cells, resulting in thousands of geneticin-resistant cell clumps (transclones). Transformation rates varied with genotype and construct. In one genotype, the number of transclones peaked at approximately 70% of the total cell clumps of target material. Transclones were further selected on the basis of morphological characteristics for embryogenicity and screened by reporter gene expression and/or molecular markers to assure stable transformation. Airlift bioreactors have enabled a great acceleration of chestnut transformation by producing high-quality embryogenic tissue in larger quantities and with lower labor and operating expense than previously used approaches.

COMPARATIVE GENOMICS OF ENVIRONMENTAL STRESS RESPONSES IN NORTH AMERICAN HARDWOODS

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The increasing incidence of introduced exotic pests, diseases and invasive plants, combined with climate change and forest fragmentation, threaten the sustainability of our forest ecosystems. The eastern hardwood forests are complex biological systems, covering over 400 million acres of bottomland and riparian sites, major watersheds, mesic sites and upland xeric sites. These forests provide habitat and food for wildlife, stabilization of riparian zones, long-term carbon sequestration and other essential ecosystem services as well as wood and biomass products for human use. Currently, few genomic resources are available for use in studying the consortium of hardwood species that compose the eastern forests. An interdisciplinary team are working together to develop new genomic resources for important species that represent the major taxonomic groups of eastern hardwood trees, from the oldest to more recently evolved, including yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), honey locust (*Gleditsia triacanthos*), northern red oak (*Quercus rubra*), black walnut (*Juglans nigra*), sugar maple (*Acer saccharum*), blackgum (*Nyssa sylvatica*), and green ash (*Fraxinus pennsylvannica*). The project will produce sequence databases for expressed genes, genetic markers, genetic linkage maps, and reference populations This will provide lasting genomic and biological resources for the discovery and conservation of genes in hardwood trees for growth, adaptation and responses to environmental stresses such as drought, heat, insect pests and disease.

COMPARISON OF SHORTLEAF X LOBLOLLY PINE F1 HYBRID PHYSIOLOGY AND MORPHOLOGY TO PARENT OPEN-POLLINATED OFFSPRING

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Hybrids between shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) previously were more frequent in drought and fire prone areas west of the Mississippi River (Hare and Switzer 1969; Edwards-Burke and Hamerick 1995; Tauer et al. 2007; Raja et al. 1997; Stewart et al. 2011 unpublished). However, recent evidence indicates that hybrids have been increasing at an alarming rate since the 1950's throughout the southeastern US (Stewart et al. 2011 unpublished). The goal of this study was to compare the physiology and morphology of artificial hybrids to those of the parent populations to determine whether shortleaf pine x loblolly pine hybrids might inherit useful traits from their parent species that have allowed them to thrive and increase in abundance over the past 60 years.

Materials and Methods

We examined several morphological and physiological characteristics of shortleaf pine (six families), loblolly pine (six families), and their hybrid seedlings (12 crosses) originating from the Western Gulf region. Seedlings were grown in a raised-bed nursery in Goldsby, Oklahoma in four replications of densely stocked family plots (114 seeds per 0.7 x 0.9 m plot) and in four replications of single tree plots spaced at 0.3 m x 0.3 m. During the dormant season following the first growing season, subsets of seedlings from the density plots were top-clipped and subsequent sprouting was monitored along with basal stem crooking (a fire adaptation of shortleaf pine). During the second growing season, morphological measurements on intact seedlings from the single tree plots included height, ground line diameter (GLD), needle characteristics (needle length, needle radius, needles per fascicle, fascicle sheath length, and specific leaf area). Physiological measurements on intact seedlings from the single tree plots focused on leaf-level variables, including net photosynthesis, stomatal conductance, intercellular CO_2 concentration (C_i), transpiration, ^{13}C isotope discrimination (δ^{13}_{CVPDB}‰), and foliar nitrogen concentration. Gas exchange measurements were taken with a Li-Cor 6400 (Lincoln, NE) infrared gas analyzer with an attached cuvette that controlled irradiance, temperature, CO_2 concentration, and water vapor. Measurements were taken four times over the second growing season, and data was analyzed using Proc Mixed for differences between genotype.

Results and Discussion

Shortleaf pine had the highest number of sprouts per stump (17.9 sprouts), followed by hybrid pine (15.3 sprouts), and loblolly pine had the lowest number of sprouts per stump (7.8 sprouts) ($p < 0.0001$). After the sprouting study, each stump was removed and checked for the basal crook fire adaptation typically unique in shortleaf pine: the lower stem crooks and lays parallel to the ground, pulling the dormant bud cluster (present in both loblolly and shortleaf pine) down to

ground level, keeping it better insulated from fire (Mattoon 1915). Stumps with basal crooks that ran parallel to the ground were considered 'strong' and functional crooks. Stumps that had only a slight bend were considered 'weak' and non-functional crooks. Shortleaf pine expressed higher strong crooking (42.6%) than both hybrids (6.4%) and loblolly pine (1.8%), which were not significantly different ($p < 0.0001$). Shortleaf pine is known to develop 100% strong crooking under normal field conditions within two to three months , but overly dense and shaded populations such as those in the nursery beds can delay crooking for several years (Stone and Stone 1954, Little and Somes 1956).

At the end of the second growing season, loblolly pine (105 cm) and the hybrids (105 cm) both were significantly taller than shortleaf pine (90 cm). Final GLD were similar for loblolly pine (31.8 mm) and the hybrid pine (33.0 mm) and larger than shortleaf pine (22.4 mm) ($p < 0.0001$).

Needle characteristics measured throughout the growing season on the first and second flushes indicated hybrids were intermediate between the two parent species, with loblolly pine having larger, thicker, longer, and more needles per fascicle than shortleaf pine. This is confirmed by several other shortleaf pine x loblolly pine hybrid studies (Mergen et al. 1965, Little and Righter 1965, Hicks 1973), but their use in hybrid identification should be limited due to large variation in genetic and environmental influences (Stewart et al. 2011 unpublished).

For leaf-level physiology measurements, there were no significant differences in any traits among species with the exception of C_i, ^{13}C isotope discrimination, and foliar nitrogen concentration. Loblolly pine had significantly higher C_i than shortleaf and the hybrid ($p = 0.01$), suggesting that it had a lower instantaneous water use efficiency (WUE; carbon gain per water loss). Although instantaneous WUE showed hybrids similar to shortleaf pine, the $\delta^{13}C$ discrimination indicted that the hybrid and loblolly pine had similar WUE that were lower than shortleaf pine ($p = 0.03$). These results confirm that shortleaf pine has a greater WUE than loblolly pine, and that the hybrid pine has similar or greater WUE than loblolly pine.

Loblolly pine x shortleaf pine hybrids grew fast like loblolly pine, possessed greater short-term WUE like shortleaf pine, and sprouted vigorously like shortleaf pine. This combination of positive traits exhibited by the hybrid seedlings may help explain why hybridization has increased over the last 60 years. Because shortleaf pine does not confer the basal crook fire adaptation to the hybrid, it is possible that fire suppression further enables the establishment of hybrids more readily than in the past.

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CLONING ELITE HYBRID SWEETGUM TREES FOR ENHANCED BIOMASS PRODUCTION AND OTHER APPLICATIONS

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Somatic embryogenesis-derived hybrid sweetgum (*Liquidambar styraciflua* x *Liquidambar formosana*) clones developed for pulp and paper and biomass energy applications, have displayed a range of growth rates and other phenotypic variation. Some of the fast-growing clones show promise for fiber production, while others offer potential as ornamental trees. We investigated variables to try to improve somatic seedling quality of the most interesting hybrid sweetgum clones. A pre-germination cold treatment of at least eight weeks improved both germination frequency and conversion frequency of the somatic embryos to close to 100 percent, and produced more vigorous plantlets than embryos given a four-week cold treatment or no cold treatment. Germinating embryos vertically in test tubes rather than on plates of gelled medium helped eliminate a problem with crooked root collars in the resulting somatic seedlings, which had previously been found to lead to a higher chance of stem breakage. A group of eight-year-old hybrid clones that had displayed outstanding growth rates in a test planting were propagated via somatic embryogenesis using staminate inflorescence explants excised from dormant buds. Embryogenesis induction for the three clones in the study ranged from 1.6% to 12.8%, depending on clone and plant growth regulator treatment, with NAA providing a higher induction frequency than TDZ. A demonstration planting of somatic seedlings representing seven hybrid sweetgum clones revealed a number of potentially useful phenotypes for rapid biomass production or ornamental uses after one season of growth, with some clones growing over 1.1 m in 4 months and others with dwarf or shrub phenotypes. Some clones also displayed striking fall leaf color. The manipulation of embryogenic suspension cultures of the hybrid clones will enable the synchronous production of thousands of propagules of the most desirable clones for biomass energy, as well as for landscape trees and other applications.

THE *ERF-AP2* GENE IN POPLAR STRESS PATHWAY

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Trees regulate vegetative growth in response to internal and environmental factors. While favorable conditions promote vegetative growth, stress induces growth cessation. Our research shows that the *FLOWERING LOCUS T2* (*FT2*) gene is involved in controlling vegetative growth during the growing season in poplar trees (*Populus* spp.). To understand the mechanism of how *FT2* controls vegetative growth under stress, we conducted microarray studies and identified the *Ethylene-Responsive Factor APETALA2* (*ERF-AP2*) gene, downstream of *FT2*. *ERF-AP2* belongs to a family of transcription factors that may have a number of functions in growth and development. We conducted physiological studies to understand the relationship between *FT2* and *ERF-AP2* in relation with tree growth and development. We will discuss our findings in this presentation.

REVEALING GENETIC BARRIERS AT A SMALL PROTECTED AREA FROM FINE-SCALE SPATIAL GENETIC STRUCTURE ANALYSIS OF *TAXUS CUSPIDATA* IN MT. SOBAEK, SOUTH KOREA

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Introduction

Pacific yew (*Taxus cuspidata* L.) is an endangered species at subalpine mountains and a keystone species at a forest protected area in Mt. Sobaek, South Korea. Population genetic differentiation of the yew tree at species level (Gst =0.067, Lee *et al.* 2000) was moderate and relatively lower than those of other coniferous species. Also, some amount of differentiation among sub-subpopulations within a subpopulation (Fst = 0.042, Kwon 2003) was reported using isozyme markers. In this study, we analyzed fine-scale spatial genetic structure and found out genetic boundaries at a small forest protected area of *Taxus cuspidata* using microsatellite makers.

Material and Methods

Study site

To examine the spatial genetic structure and the distribution pattern of *Taxus cuspidata*, a study plot of 1.2ha (150m×80m) was set up at a protected area in Mt. Sobaek located in the middle of South Korea. The plot was composed of 20 subplots with the size of 30m x 20m. We investigated every yew tree in the plot.

Growth performance measurement

Every individual in the plot was estimated tree height and diameter in breast height (DBH). And their habit and vitality level were classified into the modified Grzegorz et al. (2005) categories. The categories of the habit were:

1. Shrub-like,
2. Monocormic (one main trunk without ramification), and
3. Polycormic (trunk bi- or trifurcate).

The categories of the vitality and health were:

1. Sound without cavity – no visible necroses and no reduction in number of needles,
2. Poor without cavity – small necroses and/or slightly reduced number of needles on tops of main branches,
3. Sound with cavity – no visible necroses and no reduction in number of needles with cavity,
4. Poor with cavity - small necroses and/or slightly reduced number of needles on tops of main branches with cavity, and

5. Very poor-large necroses and significantly reduced number of needles on most branches.

Microsatellite analysis

Needles from 111 individual trees in the plot were sampled for extracting total DNA. We screened 61 nSSR markers from congener species and selected six primer sets (TY24, TY16, TAX86, TB31, TB50, and TB56) that gave clear and polymorphic peak-patterns for the analysis. The PCR conditions were followed by modified Mahmoodi et al. (2010) and PCR products were electrophoresis using Prism 3130*xL* Genetic Analyzer (ABI) with GeneScan-500 size standard. All alleles were cropped using GeneMapper v4.0 and then manually double-checked.

Data analysis

Estimation of genetic diversity, spatial autocorrelation and genetic surface analysis were conducted with Arlequin (Excoffier et al. 2005), AIS (Miller, 2005), GenBMap (Cercueil et al. 2007), and Barrier (Guerard 2004) programs respectively.

Results and Discussion

Growth performance of yew trees in the plot showed that the trees were evenly categorized into three tree types as the habit and the tree vitality and health were classified into 84% of good condition. Average of tree height and diameter in breast height (DBH) were 4.9m (±3.1) and 32.9cm (±22.9) respectively. Genetic diversity indices as number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity were 3.72, 0.364 and 0.680, respectively (Table 1).

These values were little bit high taking restricted seed dispersal distance within a small stand of *Taxus cuspidata* into consideration. Spatial autocorrelation revealed that individuals in the plot were genetically homogeneous within approximately 20m of distance and randomly distributed from 20m to 50m of distance (Figure 2). Therefore, the results suggest that individual selection for *ex situ* conservation or the study on population genetic diversity in *Taxus cuspidata* should be made with at least 20 meters of spatial interval between samples.

Genetical bandwidth mapping (Cercueil *et al*. 2007) showed that the area of yew trees were separated by brown or white colored zones indicated intermediate or high values of genetic differences among individuals, respectively (Figure 3). Those colors represented the potential genetic barriers. Green colored zone in the map comes from less genetic information due to absence of yew trees. To verify more clearly the genetic barriers, Monmonier's algorithm was used and analyzed the individual genetic configuration. Red lines in the map were the genetic barriers following the algorithm. On the basis of the genetic barriers, the plot was divided into three genetic boundaries and 18% of total genetic variation from the AMOVA(Analysis of Molecular Variance) could be explained with the 'hidden' genetic structure. This study showed being of genetic barriers within a small forest protected area and it could help to make a decision for *in situ* conservation plan of *Taxus cuspidata* in this region.

Table 1. Genetic diversity indices of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea.

Locus	N	Na	Ne	I	Ho	He	F
TY24	100	12	5.2	1.896	0.130	0.807	0.839
TY16	99	5	3.6	1.411	0.747	0.720	-0.038
TaX86	95	8	3.1	1.404	0.305	0.673	0.546
ABR Ⅱ-TB31	87	3	1.9	0.768	0.218	0.485	0.549
ABR Ⅱ-TB50	82	13	6.4	2.048	0.634	0.843	0.248
ABR Ⅱ-TB56	96	3	2.2	0.885	0.146	0.552	0.736
Mean	93.2	7.3	3.72	1.402	0.364	0.680	0.480

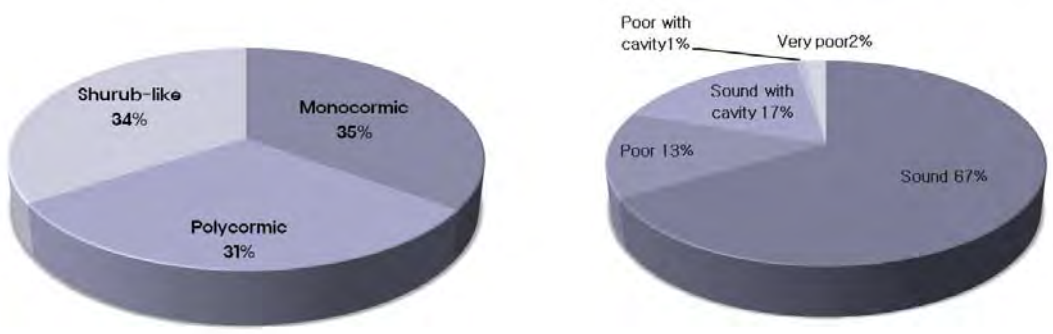

Figure 1. Proportion of tree form and growth performance of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea.

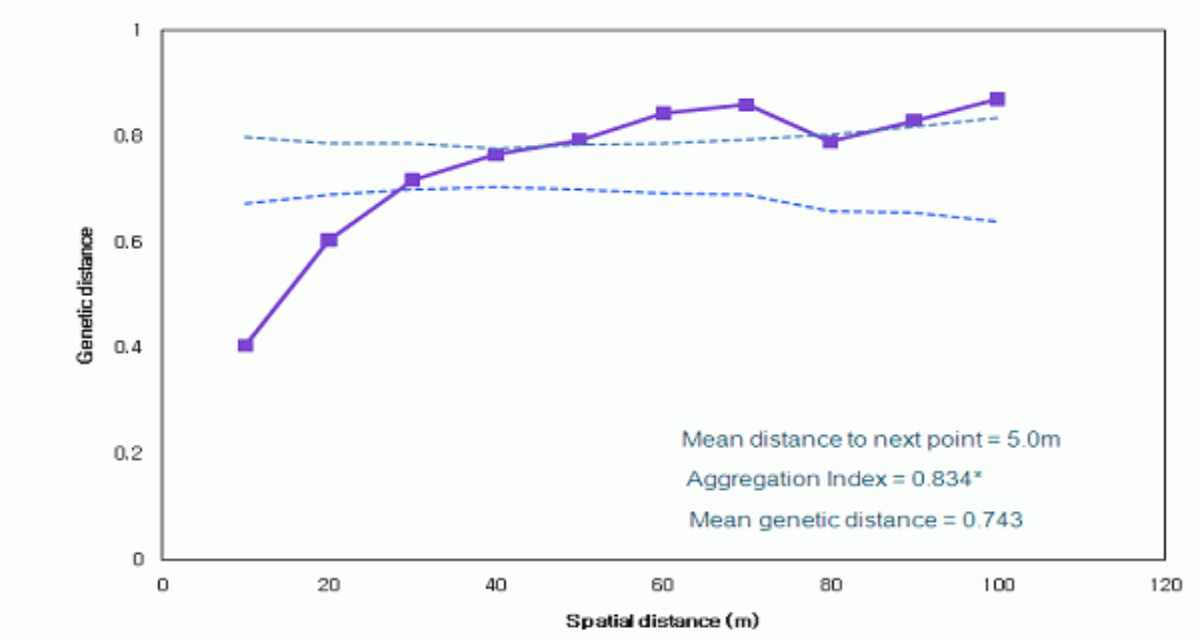

Figure 2. Correlogram of *Taxus cuspidata* at the study plot in Mt. Sobaek, Korea. The solid line and two spotted lines showed spatial autocorrelation and the upper and the lower confidence intervals as 95%, respectively.

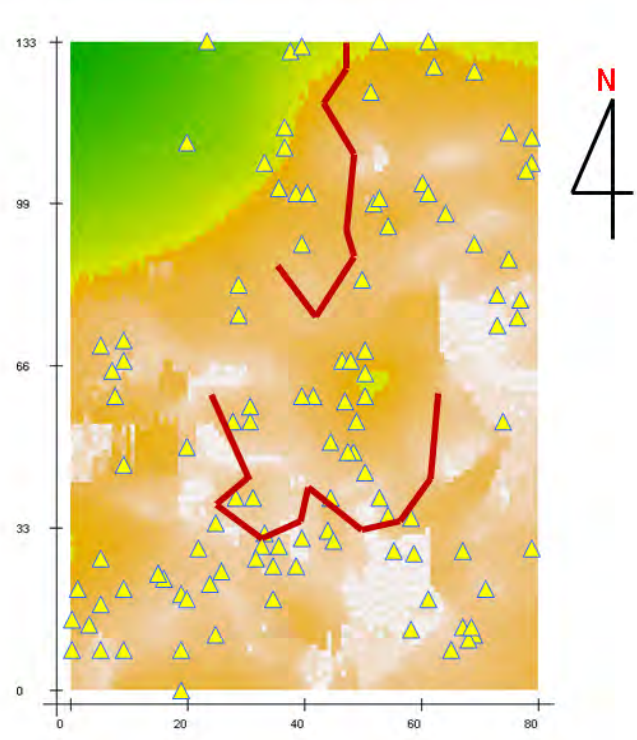

Figure 3. Genetical bandwidth map at the study plot of *Taxus cuspidata* in Mt Sobaek, Korea.

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IDENTIFICATION OF MARKERS LINKED TO *AVR1* IN *CRONARTIUM QUERCUUM* F. SP. *FUSIFORME* USING A NOVEL NEXT-GENERATION SEQUENCING APPROACH

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Fusiform rust disease, caused by infection of the fungal pathogen *Cronartium quercuum* f.sp. *fusiforme*, produces galls on stems and branches of southern pines. Stands of susceptible genotypes are often poorly stocked because stem galls weaken stems and make trees susceptible to lodging. Gene-for-gene interaction between the *Pinus taeda* resistance gene *Fr1*, and the corresponding pathogen avirulence gene *Avr1* has been documented in previous work (Wilcox et al. 1996; Kubisiak et al. 2011). Obtaining markers for avirulence loci would allow pathogen populations to be surveyed where plantations were to be established, enabling growers to plant trees with corresponding resistance genes that ensure the stand is resistant to rust. We previously showed that such markers could be identified because the gene-for-gene relationship acts as a "sieve" for avirulence alleles when a heterozygous fungal culture is inoculated on a resistant host. After inoculation of a susceptible tree (*fr1/fr1*), spores harboring either virulence or avirulence alleles (*Avr1* and *avr1*) persist and produce haploid pycniospores. In contrast, pycniospores from inoculated resistant trees (*Fr1/fr1*) only contain spores with *avr1*, selecting against the avirulence (*Avr1*) allele. Here we evaluate a restriction enzyme-based approach to identify markers linked to *Avr1*, using bulk segregant analysis to compare pycniospore DNA sequences obtained on a next generation sequencing platform (Illumina GAIIx). Reads present in spores from the susceptible host, but absent or significantly reduced in frequency in spores from the resistant host, are probably linked to *Avr1*. While the marker discovery approach does not rely on an assembled genome sequence, these markers we identify can be used as queries to interrogate the genomic sequence of *Cronartium quercuum* f.sp. *fusiforme*, as a strategy to integrate the *Avr1*-linked markers with the physical representation of the genome. This approach should lead to the identification of *Avr1* itself, and should foster an analogous strategy to quickly identify other avirulence genes in *Cronartium quercuum* f.sp. *fusiforme*.

IDENTIFYING PROTEIN-DNA INTERACTIONS INVOLVED IN WOOD DEVELOPMENT IN POPLAR

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Trees are unique among plants since they have extremely long life spans and have the ability to generate large quantities of woody biomass resulting from the formation of secondary xylem formed by the vascular cambium. The composition of xylem and the arrangement of cell types determine the physical and structural properties of wood. Gene-specific transcription factors (TFs) are DNA-binding regulatory proteins capable of either activation or repression by binding to a specific region of DNA, normally located in the 5-prime upstream region of the gene. These Protein-DNA interactions are responsible for gene expression during plant growth and development. One method for altering lignin composition is by manipulation of TFs involved in lignin biosynthesis. By using data from tissue specific *Populus trichocarpa* micro-arrays, TFs expressed 4 fold or greater in a xylem-biased manner are being used to screen for interactions with specific promoter sequences. The 1700-bp promoter region for the lignin biosynthesis enzymes caffeic-acid methyl transferase (COMT), and hydroxycinnamoyl transferase (HCT) were screened against a mini-library composed of 40 xylem-biased TFs in a Gateway-Compatible Yeast One-Hybrid (Y1H) assay to screen for Protein-DNA interactions. Results indicate transcriptional regulation by TFs from both the MYB and Zinc-finger families are involved in regulation of lignin biosynthesis. Subsequent transient assays in *Nicotiana benthamiana* have resulted in confirmation for some of these interactions based upon reporter GUS staining level when compared to background. Novel interactions are currently being characterized *in planta* by ectopic expression and RNAi mediated gene-silencing.

RANGEWIDE ASSESSMENT OF GENETIC STRUCTURE AND VARIATION IN EASTERN HEMLOCK (*TSUGA CANADENSIS*), AN IMPERILED CONIFER, USING MICROSATELLITE MARKERS

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Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is an ecologically important species experiencing extensive mortality caused by the invasive hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand). We conducted the first rangewide molecular marker population variation study for eastern hemlock to **(1)** help guide gene conservation efforts for the species, **(2)** assess the genetic effects of isolation on peripheral disjunct populations, and **(3)** better understand the biogeographical processes that shaped the genetic architecture of the species. This study encompasses 60 eastern hemlock populations, of which eight are disjuncts, half are from areas north of the maximum extent of the Wisconsinian glaciation, and 38 are in counties infested with HWA. We used 13 highly polymorphic and consistent nuclear microsatellite loci in the analysis after screening 42 loci isolated from eastern hemlock, Carolina hemlock (*Tsuga caroliniana* Engelm.) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.).

We found moderate levels of genetic differentiation among populations, with approximately 6 percent of the variation among, rather than within, populations. Overall observed heterozygosity was less than expected heterozygosity, and most populations had relatively high levels of inbreeding. The species appears to have two main centers of genetic variation, in the southern Appalachians and in New England. Unique alleles, however, were present in the highest numbers in western populations, including disjuncts in Indiana and Kentucky. Interior populations were more significantly more diverse than disjunct populations by nearly every measure. Disjuncts, however, on average had more private alleles. Levels of genetic variation in Northern and Southern populations were not generally significantly different, although Southern populations were, on average, significantly more inbred than northern populations. There was little difference in genetic variation between populations in counties infested and uninfested by HWA, although uninfested had more unique alleles on average.

A spatially explicit Bayesian clustering analysis of individual trees using TESS suggested that the species contains five gene pools. Strong geographic patterns were apparent in the arrangement of these gene pools, with all five gene pools present in southern populations, and only one dominant in northern populations. These findings suggest that one or more Pleistocene glacial refuge existed in the South, with a main post-glacial movement first into the Northeast and then into the Great Lakes region. A separate migratory path may have existed to the populations west of the southern Appalachian crest.

The results suggest the following: **(1)** Efforts to conserve the genetic variation of eastern hemlock should focus on the areas with the highest allelic richness and heterozygosity (the

southern Appalachians and New England) and on other areas with high numbers of unique alleles. **(2)** Gene conservation activities also should target disjunct populations. While these are more inbred and less genetically diverse, several also contain high numbers of unique alleles or represent gene pools rare in the interior of the range. **(3)** It is not too late to conserve eastern hemlock genetic variation, given that much genetic variation exists in locations that have not yet been impacted by HWA.

GENOMIC SELECTION IN LOBLOLLY PINE

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Genomic Selection (GS) is a method of predicting the genetic value of an individual based on high-density marker data. The application of GS in early selection is particularly valuable for long-lived, perennial tree species. We report the first assessment of the utility of GS in a forest species, the conifer *Pinus taeda* (*Loblolly pine*). A set of 800 individuals, clonally replicated in four sites, were genotyped with 3983 SNPs and phenotyped for Diameter at Breast Height (DBH) and total height (HT). Prediction models were developed using a Genomic BLUP (Best Linear Unbiased Prediction) procedure. The prediction models were validated in all four sites and across different ages and the accuracy of those models was calculated. GS model accuracies remained high across environments within the same breeding zone. However, models generated at early ages did not perform well to predict phenotypes at age 6. The selection efficiency per unit time is 53–118% higher using GS compared to phenotypic selection, assuming a conservative reduction of 50% in the length of the breeding cycle. These results demonstrate the feasibility and remarkable gain that can be achieved by incorporating GS in breeding program of conifers and long-lived perennial species.

GENETIC VARIATION AND CONTROL OF ANATOMICAL, CHEMICAL AND MECHANICAL WOOD PROPERTIES OF JUVENILE WOOD IN LOBLOLLY PINE

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Wood cores were collected from 3800 4-year old loblolly pine (*Pinus taeda*) trees from 2 sites in the CCLONES trail (Baltunis et al., 2007). Core wood density and latewood percentages were determined with x-ray densitometry, C6 and C5 carbohydrate as well as lignin contents of rings 3 + 4 were determined with pyrolysis molecular beam mass spectrometry, and wood velocity stiffness was measured both in isolated cores and in-trees in the field. Interestingly, substantial phenotypic variation was observed for wood properties. Wood lignin content in rings 3 + 4, varied from 21.3 to 35.6%. Wood carbohydrate peak height varied about two-fold for 5 and 6 carbon sugars and 5 plus 6 carbon sugars combined. Velocity stiffness ranged from 2.2 to 12.1(km^2/s^2) measured in standing trees in the field and 1.8 to 21.4 (km^2/s^2) measured in the lab with dried 5 mm increment cores. Specific gravity (SG) varied from 0.184 to 0.518 for whole cores, 0.337 to 0.498 for year 3 and 0.229 to 0.554 for year 4. Latewood and earlywood SG ranged from 0.352 to 0.677 and 0.165 to 0.444 for year 3 and from 0.289 to 0.748 for latewood and 0.130 to 0.454 for earlywood in year 4. Latewood percentage varied from 1.68 to 53.32 in year 3 and 1.44 to 66.82 in year 4. Genetic parameters - clonal repeatability, narrow sense heritability, additive, dominance and epistatic components – and pairwise genetic correlations were computed and will be reported for all wood properties.

PERTURBATION OF TUBULIN EXPRESSION AND POSTTRANSLATIONAL MODIFICATIONS IN *POPULUS*

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Microtubules form part of the cytoskeleton and are involved in many dynamic processes, including cell wall deposition. Alpha- and beta-tubulin monomers polymerize into microtubules and these monomers are encoded by multi-gene families in *Populus*. An unusually high ratio of beta-to-alpha tubulin gene family members distinguishes *Populus* from many other plant species. In addition, a high degree of C-terminal sequence variability is predicted for *Populus* tubulins. This variation may impact various posttranslational modifications (PTMs) known to occur at or near the C-terminus of animal tubulins. In *Populus*, alpha- and beta-tubulins show differential expression patterns and a small subset of genes is preferentially expressed in xylem. We are investigating the roles of this subset of xylem-abundant tubulins during plant growth and development using a transgenic approach. Alpha- and beta-tubulin genes were transformed into *Populus* in various combinations. Genes encoding PTM mimics of alpha-tubulin were also included. The transformation and regeneration efficiency was very low in several rounds of transformation attempts, and the majority of the transformants failed to develop normally. Interestingly, successful regeneration of transgenic plants was obtained only with PTM mimics. Transgenic trees were grown and monitored in the greenhouse. Transgenic plants exhibited altered leaf expansion, which led to greater width-to-length ratios than in wildtype, and in many cases, mature leaves exhibited abnormal curling. The most severe curling occurred in transgenic lines where bark and wood of mature stems was lighter colored than in wild-type. Bark and wood tissues were subjected to various analyses, including metabolic profiling, gene expression, lignin analysis, microfibril angle and wood density. Results from the transgenic experiments indicate that it is possible to engineer novel expression of specific alpha- and/or beta-tubulins to have an effect on growth and wood properties of trees.

MOLECULAR CHARACTERIZATION OF A *CINNAMYL ALCOLHOL DEHYDROGENASE (CAD)* GENE FAMILY IN *LIRIODENDRON TULIPIFERA* L., A BASAL ANGIOSPERM SPECIES

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Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in lignin biosynthesis since it catalyzes the final step in the synthesis of monolignols. The *CAD* gene family has been extensively studied in several species, including *Arabidopsis thaliana*, *Oryza sativa*, and *Populus*. However, its role in basal angiosperm species and relationship with modern plants still remain unknown. In this study, we report this gene family in a basal angiosperm species, *Liriodendron tulipifera* L., which is an important timber tree species with great evolutional, ecological and economic values. We identified seven *CAD* family genes in *Liriodendron tulipifera* from a comprehensive EST dataset built from ten tissue types. The phylogenetic analysis grouped one of the *Liriodendron CAD* genes in Class I, one in Class III and the remaining five in Class II. qRT-PCR analysis showed that they have distinctive expression patterns in different tissue types, such as xylem, root, bud and leaf. *LtuCAD1* (Class I, *bona fide CAD*), predominantly expressed in xylem, was able to partially recover the lignin content loss in the *Arabidopsis CAD4/5* double mutant, suggesting that it is highly possible that *LtuCAD1* serves as a primary *CAD* gene involved in lignification. The intensive study of *CAD* gene family in *Liriodendron tulipifera* will not only broaden the knowledge of lignin evolution from the ancient gymnosperm to angiosperm, but also provide a new hotspot for manipulation of lignin biosynthesis in woody plants to facilitate its large-scale production of biofuels.

MOLECULAR CHARACTERIZATION OF A HYBRID POPLAR CLONE TRANSFORMED WITH A TYROSINE-RICH CELL WALL GENE FOR IMPROVED SUGAR RELEASE

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To facilitate lignin removal and potentially advance the utilization of woody biomass as a biofuel feedstock, we previously transformed a hybrid poplar clone with a hydroxyproline-rich glycoprotein encoding gene from parsley. While our previous results suggested that the TYR transgenic plants had no significant change in total lignin content or overall morphological characteristics when compared to the wild types, a number of transgenic lines released more polysaccharides with protease digestion, and in addition were more flexible than wild type plants, as measured by storage modulus assays. In this report, gene expression studies were conducted using whole genome DNA microarrays to examine the molecular basis for the flexibility and digestion phenotypes observed in the previous study. Microarray data revealed a total of 102 differentially expressed transcripts in transgenic lines. All 102 transcripts were decreased in expression in TYR plants. The lignin biosynthetic pathway was most affected, with four genes encoding three enzymes (cinnamoyl CoA reductase, hydroxycinnamoyl-CoA transferase, and laccase) being significantly down-regulated. Transgenics also showed reduced expression of genes involved in other branches of phenylpropanoid metabolism, amino acid metabolism, and defense. Transcription factors, as well as genes involved in macromolecule catabolism, reassembly, and polysaccharide synthesis were also repressed in expression. Validation experiments by qRT-PCR indicated an average of 72% of the 20 genes chosen were down regulated in three transgenic lines used in the microarray experiments. Our results provide the first gene expression evidence for the basis of the flexible and degradable cell wall phenotype obtained in the transgenic poplar plants.

APPLICATION OF FT-NIR IN POPLAR CHEMICAL ANALYSIS

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Genetic analysis of wood chemical composition is limited by the cost and throughput of direct analytical methods. Indirect methods such as Fourier transform near infrared (FT-NIR) offer an alternative for rapid, low cost method. In FT-NIR, calibration models and their predictions are typically developed and validated from small sample sets. These models are subsequently used to estimate wood chemical composition from larger sets of new samples (e.g., Sykes et al., 2006. Ann. For. Sci. 63, 897-904). However, no direct comparison of direct and indirect estimates of wood chemical composition and the genetic parameter estimates have been reported for the same population. Here we compare for a single poplar family genetic parameter estimates obtained for wood chemical composition with data from pyrolysis molecular beam mass spectrometry (pyMBMS) and FT-NIR.

Over two thousand young greenhouse grown wood samples were analyzed for chemical composition with pyMBMS (Novaes et al. 2009. New Phytologist, 182, 878-890). We randomly selected 496 samples to build a Fourier transform near infrared (FT-NIR) calibration and validate a model based on partial least square for lignin percent, corrected lignin, G-lignin, S-lignin, S/G ratio and sugars (C5 and C6). A FT-NIR spectrometer, equipped with an X-Y stage auto-sampler was used to improve the scanning efficiency. The sample set was randomly divided into calibration (397) and prediction (99) sets. The coefficient of determination (R^2) for the calibrations ranged from 0.54 to 0.91, and the prediction model R^2 ranged from 0.46-0.88. Stronger calibration and prediction statistics were obtained with lignin compared with carbohydrates. For lignin the best prediction ($R^2 = 0.88$) was obtained for lignin percent. For carbohydrates, the strongest prediction statistics ($R^2 = 0.71$) were obtained for the m/z 144 ion which comes from cellulose. Genetic analysis of pyMBMS data and FT-NIR predictions will be compared to evaluate the utility of the indirect FT-NIR method relative to the direct pyMBMS method for parameter estimates.

PARTICIPATORY FOREST TREE IMPROVEMENT AND CONSERVATION

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University-Industry Cooperative Tree Improvement has been highly successful in the southern United States. Over nearly 60 years, three Cooperative programs have lead the way in developing and deploying genetically improved planting stocks for loblolly (*Pinus taeda*) and slash pines (*P. elliottii*). Much lower levels of success have been achieved for species of lesser economic importance such as longleaf (*P. palustris*) and shortleaf pines (*P. echinata*) and the many southern hardwoods. For American chestnut (*Castanea dentata*) the American Chestnut Foundation's breeding program is finding success with a nearly 30 year sustained effort now beginning to yield putative disease resistance planting stock in numbers for operational testing. The program has relied on funding from individuals with a passion for returning chestnut to the forest and additional sources where and when available. TACF's program consists of a breeding farm in the central part of the American chestnut range that's responsible for early generation crossing and testing and a network of state chapters that handle later generations using local American chestnut germplasm for crossing and local environments for disease screening. In addition all entities are committed to developing seed orchards for the production of planting stock for their local areas. At the same time many other tree improvement programs have come and gone while the need for genetically improved trees of many species is increasing. Participatory plant breeding is a potential means for establishing and maintaining genetic improvement programs for species of intermediate economic value. Doing so will have a large overall economic impact and positive effect on our rural and urban environments. We will describe such a tree improvement approach for forest trees in the southeastern U.S. using longleaf pine as an example. It is hoped that the discussion will stimulate a collaborative effort to initiate such a program for longleaf pine and several other forest tree species.

A MULTIPLE-SPECIES ASSESSMENT OF RELATIVE GENETIC DEGRADATION RISK FROM CLIMATE CHANGE AND OTHER THREATS

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Climate change is expected to pose a threat to the viability of forest tree species, which may be forced either to adapt to new conditions or to shift their ranges to more favorable environments. Changing climate conditions and threats of pest and pathogen infestation will increase the risk that forest trees could experience population-level extirpation or widespread decreases in productivity during the next century. In the face of these challenges, it will be important to safeguard existing adaptedness within species and to create conditions conducive for future productivity and evolution. Forest tree species, however, differ in their physiological tolerances, life-history strategies, and population dynamics. These differences could drive wide dissimilarities among species in their potential responses to changing climate conditions and other threats, and could affect the likelihood that particular species might be useful sources of biomass for energy production. Diverse management and genetic conservation strategies will be needed to ensure successful regeneration and restoration of species with differing characteristics and susceptibility to various threats. This will be a particular challenge in species-rich regions such as the Southern Appalachian Mountains of the southeastern United States. To facilitate the effective use of limited resources, we developed the Forest Tree Genetic Risk Assessment System (ForGRAS), which ranks the predisposition of forest tree species to genetic degradation, based on ecological and life-history traits, species-specific projections of climate change pressure, and predictions of pest and pathogen susceptibility. We then applied ForGRAS to 131 tree species native to the Southern Appalachians. This framework serves as a tool for planning management activities and conservation efforts, for evaluating species' genetic resources, and for detecting species' vulnerabilities. It has the advantage of accounting for multiple threats that may result in the most severe genetic impacts. The flexibility of ForGRAS allows for its application at multiple scales and across any area for which appropriate data exist for the species of interest.

GENETIC CONSERVATION OF TABLE MOUNTAIN PINE (*PINUS PUNGENS*) IN THE SOUTHERN APPALACHIAN MOUNTAINS BY THE USDA FOREST SERVICE AND CAMCORE

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The USDA Forest Service National Forest System in the Southern Region (R-8) and the Camcore program in the Department of Forestry and Environmental Resources at N.C. State University are collaborating on a three-year project to conserve the genetic resources of Table Mountain pine (TMP, *Pinus pungens* Lamb.) in the southern Appalachian Mountains. Occurring from central Pennsylvania to northern Georgia, TMP typically occupies dry, thin, nutrient poor soils on south and west facing ridges from 300 to 1200 meters elevation. Over the past several decades, populations of this fire-adapted species have declined due to wildfire suppression programs and periodic outbreaks of the southern pine beetle (*Dendroctonus frontalis* Zimm.), resulting in reduced diversity and impairing ecosystem services provided by these ridge top environments. The objectives of this project are to: (1) make genetically representative seed collections from surviving TMP populations distributed across the southern Appalachian region, (2) establish TMP seedling seed orchards/conservation banks on the existing USFS seed orchards in the southern region, (3) place seeds in long term cold storage for use in region-wide reforestation efforts, and (4) use molecular markers to describe the population genetic structure and diversity of the species. The genetic material conserved through this collaborative effort will have a critical role, along with silvicultural methods for prescribed fire to promote stand regeneration, in the restoration and rehabilitation of TMP ecosystems in the southern Appalachian region. Our paper presentation will provide an update on progress made during the first year of the project and provide a timeline of activities to occur during years two and three.

A DNA MARKER RESOURCE FOR LONGLEAF PINE GENETICS

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Microsatellite DNA markers, also known as SSR (simple sequence repeat) markers, are an essential genetic resource for population assessment, conservation genetics research, and seed orchard monitoring. We are developing longleaf pine SSR markers by evaluating mapped markers from loblolly pine and *de novo* markers from DNA sequence databases. Hundreds of potential markers are being evaluated with the goal of selecting a set of highly informative markers for use in a variety of applications. We will present our progress, research plans, and public distribution outlet for marker data.

GENETIC VARIATION OF JAPANASE ELM (*ULMUS DAVIDIANA* VAR. *JAPONICA)* EMPLOYING ISSR MARKERS

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We studied genetic variation of Japanese elm calling medicinal tree, *Ulmus davidiana* var. *japonica* (Rehder) Nakai, to establish the strategy for the conservation of its genetic resources. A total of 171 individual samples were collected from seven populations and their DNAs were used for inter simple sequence repeat-PCR amplification with 47 ISSR primers. Eight out of the 47 primers were selected and yielded 55 clear fragments to be scored. The percentage of polymorphic loci (*P*) ranged from 87.27% to 98.18% with a mean of 93.25%. The gene diversity (*h*) averaged over all was 0.33. The Shannon's information index (*S.I.*) ranged between 0.455 and 0.515 with an average of 0.494. The AMOVA showed that most of the genetic variation (96.1%) was allocated among individuals within populations. The dendrogram showed no clear association between the clustering of population and their geographical origin.

Introduction

Japanese elm belongs to the family Ulmaceae and the genus *Ulmus* L. and distributes in northeastern Asia including China, Japan and Korea. This tree is considered to be one of the most useful medicinal tree species because the extracts from the bark enhance splenocyte proliferation and cell viability. Furthermore, the extracts from the roots have an activity to prevent reactive oxygen species in human cells. In spite of these useful effects, to our best knowledge, only a few genetic studies using molecular markers have been performed for this species. The objectives of this study are to investigate the genetic variation of Japanese elm and to provide fundamental information for the conservation of its genetic resources.

Materials and Methods

Plant materials of the 171 individuals were collected from seven natural populations, *Ulmus davidiana* var. *japonica,* in South Korea (Figure 1). The genomic DNA was extracted from 80㎎ leaf using DNeasy plant mini kit. PCR amplification was performed with seven primers out of 47 UBC ISSR primers. For each primer, amplified fragments with the same molecular weight (bp) were recovered as present (1) or absent (0), and the resulting binary matrix was used in the statistical analysis. The computer program POPGENE version 1.31 was used to estimate genetic diversity parameters (Yeh et al. 1999) and Shannon's index (Table 2). The analysis of molecular variance (AMOVA) was carried out to measure the degree of genetic differentiation. A dendrogram was derived from the unweighted-pair group method with arithmetic mean (UPGMA) clustering based on basis of pair-wise Manhattan distance (Wright 1978)

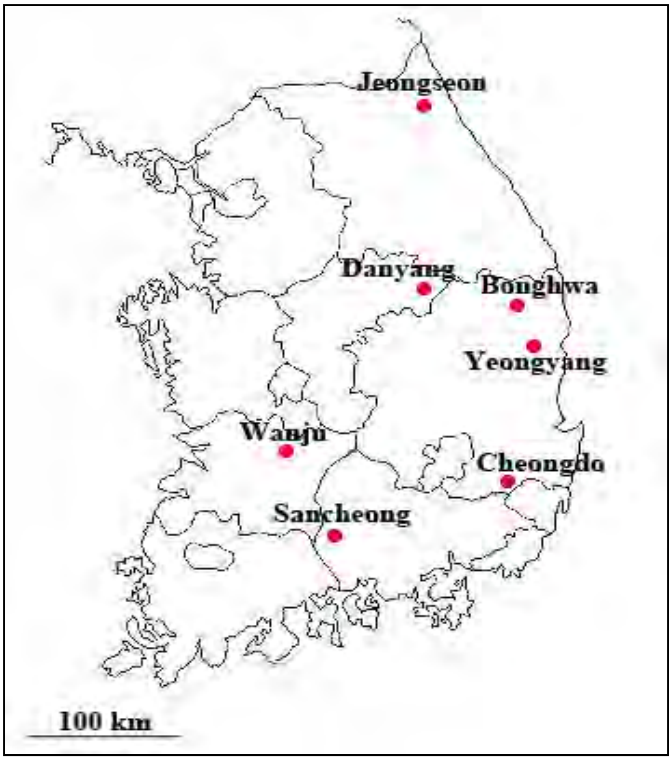

Figure 1. Natural distributions of population of *Ulmus davidiana* var. *japonica.*

Results and Discussion

The average number of the loci per primer was 6.9. Nevertheless the primer UBC #822 had only 2 bands to score, they were scored because of their clear patterns. Two markers such as UBC #807 and UBC #811 had 10 scored bands. The markers usually had (AG) or (GA) motifs (Table 1). The results from analysis of genetic variability to seven populations, Japanese elm, the Shannon's information index (*S.I.*) indicating genetic diversity ranged from 0.455 to 0.519, with an average of 0.494 at the population level (Table 2). These degrees of genetic diversity were higher than other deciduous trees such as *Oplopanax elatus*, its degree of genetic diversity was 0.187 (Lee et al. 2002) in Korea and *Kirengeshoma palmate*, its degree of genetic diversity was 0.259 (Zhang et al. 2006) in China. This is because of that endangered plant species have low genetic diversity due to the genetic drift and gene flow (Karron 1991).

Table 1. ISSR markers used for genetic variation analysis.

Primer name	Repeat region 5' → 3'	Scored bands
UBC807	$(AG)_8T$	10
UBC810	$(GA)_8T$	6
UBC811	$(GA)_8C$	10
UBC812	$(GA)_8A$	7
UBC813	$(CT)_8T$	7
UBC822	$(TC)_8A$	2
UBC855	$(AC)_8YT$	6
UBC873	$(GACA)_4$	7
Total	8	55

Y=C or T

Therefore, Japanese elm's genetic variation is higher than that species. However, other tree species showed similar or slightly lower genetic diversity such as Smile rosebay was 0.395 (Hong *et al*., 2003), *Taxus cuspidata* was 0.478 (Kwon et al., 2002), Japanese red pine was 0.453 (Hong et. al., 2007), *Camellia sinensis* had 0.343 (Yang *et al*., 2010), and *Torreya nucifera* was 0.353 of biodiversity (Hong *et al*., 2000). This result means that Japanese elm has been adapting similar environment changes with these tree species. Sancheong was the highest at 0.492 in Shannon's index (*S.I.*), while Wanju was the lowest at 0.455. But there was no significant difference among populations.

Table 2. Genetic variability of *U. davidiana* var. *japonica* from ISSR analysis.

Population	*N*	A_o	A_e	*h*	*P* (%)	*S.I.*
Bonghwa	23	1.946 (0.229)	1.571 (0.336)	0.330(0.161)	94.55	0.492(0.210)
Cheongdo	18	1.909 (0.290)	1.587 (0.321)	0.340(0.155)	90.91	0.503(0.207)
Danyang	26	1.982 (0.135)	1.573(0.2830)	0.342(0.128)	98.18	0.515(0.160)
Jeongseon	25	1.964 (0.189)	1.535 (0.310)	0.320(0.146)	96.36	0.485(0.186)
Sancheong	25	1.946 (0.229)	1.622 (0.331)	0.353(0.158)	94.55	0.519(0.206)
Wanju	24	1.873 (0.336)	1.522 (0.346)	0.305(0.174)	87.27	0.455(0.236)
Yeongyang	30	1.910 (0.290)	1.558 (0.332)	0.326(0.158)	90.91	0.486(0.210)
Average	24.43	1.933	1.567	0.331	93.25	0.494

N, sample sizes; A_o, observed number of alleles per locus; A_e , Effective number of alleles per locus; *h* , Nei's (1973) gene diversity; *P* , percentage of polymorphic loci; *S.I.*, Shannon's information index (1948). Standard deviations are given in parentheses.

The results of the AMOVA analysis to find out genetic structure in seven populations, Japanese elm, showed, 96.16% of total genetic variation that exists in individual variations within groups and among difference of populations was 13.3% (Table 3). This genetic variation of Japanese elm was lower than *Oplopanax elatus* (Φ_{ST}=0.109, G_{ST}=0.155; Lee et al. 2002), *Camellia sinensis* L. (Φ_{ST}=0.132, Yang et al., 2010), and *Alnus hirsute* (G_{ST}=0.087; Huh and Huh, 1999) in Korea. Furthermore, White elm showed Φ_{ST}=0.290 with 20 allozyme loci analysis in Finland (Vakkari et al. 2009).

Table 3. Analysis of molecular variance (AMOVA) within/among populations.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variance
Among populations	6	119.42	0.40	3.84
Within populations	164	1654.61	10.09	96.16

In the results of AMOVA, 3.84% of total genetic variation was caused by the difference among populations and 96.16% of the others were caused by between the individuals within populations. According to this the results, for effective conservation of this genetic resources to increasing genetic diversity, it is proper that conservation by select many individuals within a population rather than select many populations for *ex-situ* conservation and conservation by select a few populations rather than select many populations for *in-situ* conservation. In order to identify relationship among populations, as the results of UPGMA cluster analysis (Figure 2) in seven populations, were grouped in three parts according to the genetic distance. The first group includes Danyang, Bonghwa, and Yeongyang population. The second group is Wanju, Jeongseon, and Sancheong population. The last group is only Cheongdo population. Based on the genetic diversity and UPGMA cluster, Danyang and Sancheong represent their group, respectively because Danyang and Sancheong population showed more genetic diversity in their groups.

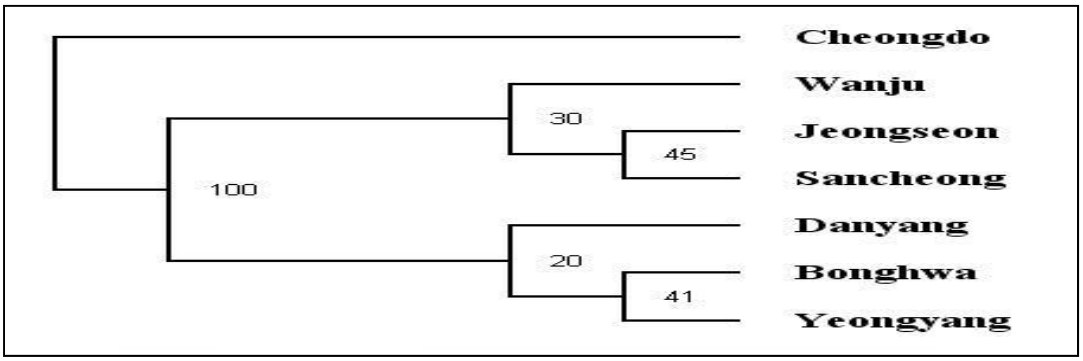

Figure 2. A dendrogram based on the Manhattan distance among seven populations of *U. davidiana* var. *japonica* generated by UPGMA clustering.

According to the above results and geographic distribution of these populations, Japanese elm (Figure 1), we found that the second group was not related to geographic distance. Because three populations within the second group were geographically distant from each other. Especially, Jeogseon and Sancheong populations are the farthest away among total populations (Figure 1) We should be more study to the origin of the species and biological evolutionary process for explain of this grouping.

Conclusions

In conclusion, in order to conserve effectively, after searching and investigating natural populations of target species, we should be decide to the optimal number and size of target species for conservation by analyzing the genetic structure and genetic variation using the appropriate genetic markers for effective conservation. Genetic studies of population on target species of conservation. In addition, these materials can be used to develop new varieties.

Acknowledgements

Financial support for this research has been provided by Korea Forest Research Institute (KFRI). We appreciate by Korea Forest Research Institute (KFRI)

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DEVELOPMENT OF A NATIONAL NARIVE PLANT GERMPLASM SYSTEM

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A high level of genetic diversity is necessary for the success of plant breeding programs and for species adaptation to changing environments. Until recently the management of genetic resources of native plants in the United States was almost exclusively conducted in the wild (In Situ). The growing impact of exotic invasive pests, weeds, and climate change has caused responsible parties to question the reliance on In Situ methods alone. More managed human-assisted methods (Ex Situ) are now needed. Therefore, elements of the USDA Forest Service have begun development of a National Native Plant Germplasm System in cooperation with the USDA Agricultural Research Service and other entities in the federal, state and private sectors. Species, techniques, objectives and data management are discussed.

LONGER BLACK WILLOW CUTTINGS RESULT IN BETTER INITITAL HEIGHT AND DIAMETER GROWTH IN BIOMASS PLANTATIONS

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Black willow (*Salix nigra* Marsh.) has the potential to be a viable biomass crop for heavy clay soils throughout the southern United States. The most favorable planting stock for woody biomass plantations is dormant unrooted cuttings, because they are easy to plant and use of clonal material allows for advancing genetic improvement. The objectives of this study were to determine the optimal cutting size and planting depths, characterize rooting ability, and study genetic variation that would enhance survival and growth of black willow. We examined four cutting diameters, three cutting lengths, and three planting depths. There were no significant age-one survival differences among the various factors. Significant age-one total height differences were shown for cutting length, depth of planting, and cutting diameter. Height was greater for those cuttings that were the longest but planted at a shallower depth. While cutting diameter was a significant factor for age-one height it followed no cutting size trend. The high survival of black willow is strongly correlated to its prolific rooting ability. There is genetic variation among region specific stands and clones.

GENETIC DIVERSITY, PHYSIOLOGIC EXPRESSION, AND CARBON DYNAMICS IN LONGLEAF PINE AT THE HARRISON EXPERIMENTAL FOREST

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In 1960, an experiment was established on the Harrison Experimental Forest in southeast Mississippi to compare productivity of planted longleaf, loblolly and slash pines of local origin (Smith and Schmidlting 1970). Longleaf pine lagged in productivity during the early years, but eventually surpassed loblolly and slash pine. Hurricane Katrina (August 2005) left the experiment heavily damaged; especially the loblolly pine plots. Recently region-wide interest in restoring longleaf pine has developed, with an important goal being to increase forest resilience to climate change and extreme climate events. Little is known about the value and variability of adaptive traits in longleaf pine. Our goal is to better understand genetic control of these traits (survival, disease resistance, productivity) as expressed on a hurricane prone longleaf pine site characterized by relatively low soil fertility. This new installation will allow a direct comparison of longleaf pine families originating from four seed sources (ranging from southeast Texas to north Alabama) under three planting densities. Physiologic differences between and within the sources will be analyzed along with differences in height, diameter, stem taper and carbon allocation to specific components (foliage, branches, stems, roots) across the planting density gradient. Allelic states of several genes will be related to survival and performance traits. This experiment will inform development of genetic guidelines for restoring resilient longleaf pine ecosystems.

Previous Experimental Results

Key findings thus far:

- Age 9, intensive culture increased productivity of all species; loblolly pine had greater height and volume than longleaf or slash pine (Schmidtling, 1973). Mean yields in the highest fertilizer treatment were loblolly 41 Mg ha^{-1} (18.3 ton $acre^{-1}$) slash 29 Mg ha^{-1} (12.9 ton $acre^{-1}$), longleaf 12 Mg ha^{-1} (5.4 ton $acre^{-1}$).
- Age 25, longleaf surpassed slash and loblolly pine in height in the control plots, characterized by low nutrient availability. At the highest level of intensity loblolly was still >2 m taller than the other species (Schmidtling, 1986).
- Age 39, there were no species differences in diameter at breast height (dbh), though dbh increased with intensity level. Significant differences in height were attributed to both species (slash>longleaf>loblolly) and cultural intensity (3x fert.>2x fert.>1x fert>control>disking no fert).
- Age 45, Hurricane Katrina impacted the site; longleaf pine suffered the least mortality, followed by slash and loblolly pine respectively (7%, 15%, 26%) (Johnsen et al. 2009).
- In 2006, after Hurricane Katrina, mean basal area across all treatments was 23 m^2 ha^{-1} (100 ft^2 ac^{-1}) for longleaf, 12.4 m^2 ha^{-1} (54 ft^2 ac^{-1}) for loblolly, and 19.3 m^2 ha^{-1} (84 ft^2 ac^{-1}) for slash pine.

New Experiment

The new installation will compare four longleaf pine sources (ranging from southeast Texas to north Alabama) under three planting densities (746, 1329, 2197 trees per hectare or 303, 528, 889 trees per acre) using a randomized complete block design. Within each plot, there will be four genetic source split-plots: USFS Region 8 (R8) TX/LA source, R8 south MS/south AL source, R8 north AL source, and an unimproved local source (i.e., control, representing genetic quality of original planting). Each R8 source represents one generation of genetic improvement. Physiologic differences between and within the sources will be analyzed along with differences in height, diameter, stem taper and carbon content. Allelic states of several genes will be tested relative to survival and performance traits. Experiments such as this will inform development of genetic guidelines for restoring resilient longleaf pine ecosystems.

Discussion

The original experiment has been invaluable for comparing long term productivity and carbon dynamics between three species of planted pines and continues to have demonstration and research value. Instead of simply harvesting the entire site and starting over, a novel plan which includes retaining some of the original plots and moving them to uneven age management with thinning was devised. Natural regeneration of longleaf pine is most successful in large gaps in the canopy. We are installing each of the new measurement plots in 55 m by 55 m gaps (180 ft by 180 ft) created by clear cutting (Figure 1). Some of the original longleaf pine plots have accrued exceptional basal area, with a few plots approaching 45 m^2 ha^{-1} (196 ft^2 ac^{-1}); 11 plots will be thinned to 23 m^2 ha^{-1} (100 ft^2 ac^{-1}) to continue studying them under relatively high density (Figure 1). The rest of the plots will be thinned to 14 m^2 ha^{-1} (60 ft^2 ac^{-1}). Prescribed fire will be re-established on a 2 year cycle.

Several goals will be accomplished by this study: 1) creation of a longleaf pine planting density x genetic source study, 2) restoration of a longleaf pine ecosystem with fire and planting, 3) enhanced aesthetics and habitat with gap layout, 4) unique opportunity to study longleaf genetics and physiology at the Harrison Experimental Forest in a multi-age stand, and 5) initiation of a genetic field study for genetically mapping quantitative traits in longleaf pine. Thinning and harvesting are planned for summer 2011, site preparation in fall 2011, followed by planting during the winter of 2011-2012.

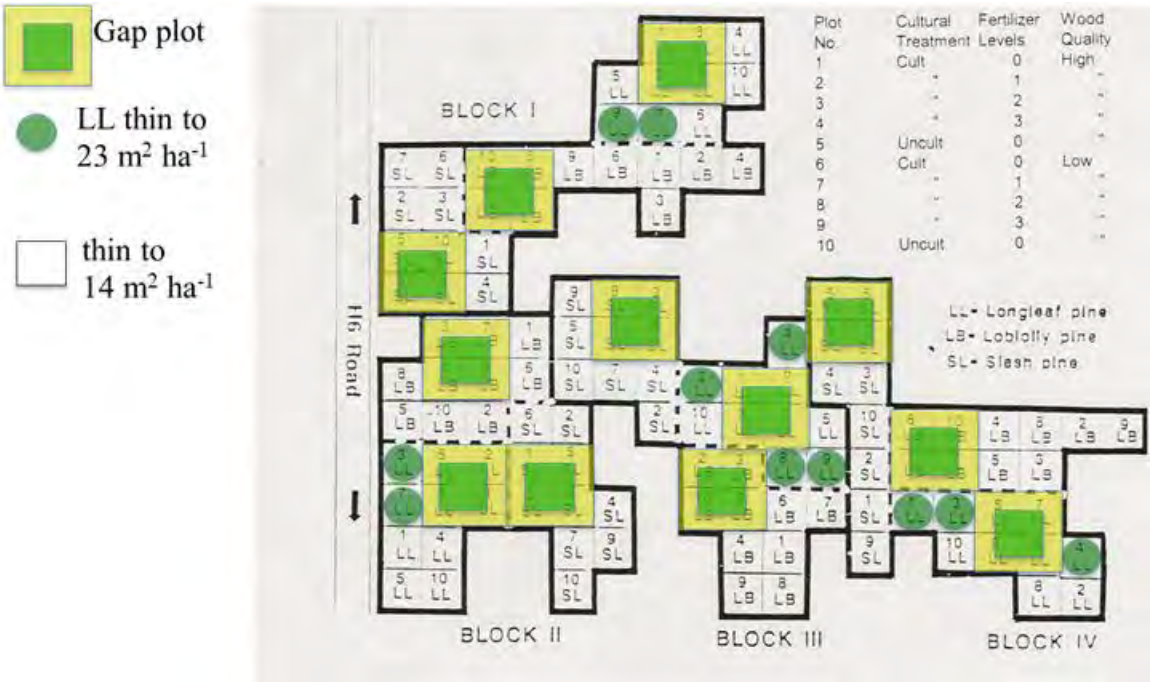

Figure 1. Map showing the location of 12 new gap plots created by combining 4 adjacent plots from the original experiment. A 5 tree buffer (yellow, light gray) will surround the new measurement area (green, darker shade). The buffer area will be thinned to14 m^2 ha^{-1} (60 ft^2 ac^{-1}) while a 55 m by 55 m (180 ft by 180 ft) area will be clear cut to create the new measurement plot. The location of 11 longleaf plots which will be thinned to 23 m^2 ha^{-1} (100 ft^2 ac^{-1}) are marked with a green (darker shade) circle, all other plots will be thinned to 14 m^2 ha^{-1} (60 ft^2 ac^{-1}).

Acknowledgements

This study is a partnership with the DeSoto National Forest. Without their vast knowledge and able assistance this project would not be possible. Special thanks to Ronald Smith, District Ranger, James Mordica, Ecosystem Restoration Coordinator, and Larry Lott, Harrison Experimental Forest Lead Forestry Technician.

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IMPUTING MISSING GENOTYPES USING NUMERATOR RELATIONSHIP MATRIX

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Genotyping does not work for all samples for all markers, so genetic data from a lab might have many missing genotypes. Yet, predictions of genetic merit of trees across markers require complete genotyping information or gene content. Excluding individuals with missing genotypes is not desired, since it will reduce the number of individuals in the population considerably, thus reducing the power of association of markers and traits. It is therefore important to use efficient statistical methods to accurately impute missing genotypes. Human geneticists rely on genetic maps and linkage disequilibrium (LD) information from nearby markers to replace missing genotypes. Their algorithms rely on known map positions for the SNPs. Since completely sequenced reference genomes are available for only two forest tree species, methods developed by human geneticists do not work well for most forest trees.

Gengler et al. (2007) described a method to impute missing genotypes using mixed linear models and BLUP. We determined the effect on accuracy of BLUP estimated breeding values of imputation with different levels (10%, 20%, 40%, 60% and 80%) of missing genotypes. Analyses were conducted both with empirical data (3461 SNP markers in a cloned loblolly pine population of 178 genotypes) and simulated data using missing data created by random sampling (some loci missing in all individuals) or by structured sampling (all loci missing in some individuals). Simulations were used to examine the effect of family and progeny size, mating design, proportion of missing genotypes, genotyping strategy and the method for imputation on the accuracy of breeding values. Imputed genotypes were obtained using the numerator relationship matrix (the A matrix) and solving the mixed model equations of $y = Xb + Mu + e$, where **y** is the vector of gene content predictions, X is the design matrix (vector of 1s) for the mean, M is the design matrix connecting trees to the gene content vector **y**, u is the individual tree effect and e is the error variance. The solutions of mixed model equations produce predicted SNP genotypes for trees with missing genotypes. The solutions are continuous, centered on 1 because the gene content values are 0, 1 or 2.

Imputation of missing genotypes in empirical data from an unbalanced mating design with family sizes ranging from 1 to 35 was more powerful for data with structured missing genotypes at all levels of missing data than for data with random missing genotypes with same proportions of missing data. The accuracy of imputation for 10% and 80% missing genotypes ranged between 0.96 to 0.23 and 0.96 to 0.16 for structured and random missing genotypes in the data, respectively. As the proportion of missing genotypes increased in the data, the power of imputation decreased. With simulation, we found that the imputation was less affected by the distribution of missing genotypes in a balanced mating design with families of equal size. The accuracy of imputation ranged between 0.97 to 0.75 for the 10% and 80% missing genotypes in the data, respectively.

PEDIGREE CONSTRUCTION: AN ALTERNATIVE TO CONVENTIONAL BREEDING

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Abstract not provided.

GENOMIC SELECTION IN CONIFERS: STABILITY OF THE PREDICTION MODELS ACROSS SITE AND AGES

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Abstract not provided.

CONSTRUCTION OF REALIZED GENOMIC RELATIONSHIP MATRIX FOR BLUP OF BREEDING VALUES

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Genetic merit can be considered the finite sum of thousands of allelic effects, each physically located at some place on the genome, whose transmission can be traced through molecular markers. Traditionally, the BLUP of breeding values relies on average additive genetic covariances (the numerator relationship matrix **A**) derived from pedigrees to utilize information from relatives. For example, all the full-sib offspring of a cross are assumed to share 50% of genes inherited from parents. Such assumptions ignore Mendelian segregation of progeny within family. With vast advancements in marker genotyping technology and reduction in genotyping cost, it is now feasible to construct genetic covariances from markers. Dense markers are being used to trace identity by descent (IBD) at each locus and these IBD probabilities are being used to construct incidence matrices. Total genomic merit of candidates would be obtained by summing up many relevant marker effects.

Linear mixed models that utilize realized genomic relationships matrices could predict breeding values more accurately than those that use expected average genetic covariances derived from pedigrees. An approach that can be explored is the solving of mixed model equations by using the inverse of the genomic relationship matrix (**G**), in place of the **A** matrix. This may allow better estimation of individual allele effects, followed by summation across loci to obtain genomic estimated breeding values in a marker-only model.

The generation of the **G** matrix based on a set of biallelic SNP markers genotyped for a clonal population of loblolly pine and its application into calculation of accurate genomic estimated breeding values is discussed. The correlation between the genomic estimated breeding values and breeding values based on a pedigree-based model is used to determine the potential gain using markers in a forest tree breeding scenario.

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2013 Southern Forest Tree Improvement Committee - (updated July 2011)

Group A

State Forestry - 3 representatives (6-year term)	Terms	
1. Onesphore (Ones) Bitoki – Virginia Department of Forestry	2011	2017
2. Ken Roeder – North Carolina Forest Service	2011	2017
3. Diane Warwick – Tennessee Division of Forestry	2011	2017
Forest Industry - 3 representatives (6-year term)	Terms	
1. Al Lyons – Hancock	2007	2013
2. Bob Purnell – Weyerhaeuser	2011	2017
3. Josh Sherril – Rayonier	2009	2015
Forestry School - 3 representatives (6-year term)	Terms	
1. Amy Brunner – Virginia Polytechnatute Instute	2009	2015
2. Joshua Adams – University of Arkansas	2011	2017
3. Randy Rousseau – Miss. State University	2007	2013
National Association of Plant Breeders - 1 representative (6-year term)	Terms	
1. Ross Whetten – NC State University	2011	2017
Meeting Host Graduate Student - 1 representative (2-year term)	Terms	
1. Clemson University and ArborGen	2011	2013

Group B

US Forest Service - 3 representatives (indefinite term)	Terms	
1. C. Dana Nelson – Southern Research Station	Indefinite	Indefinite
2. George Hernandez/Ron Overton – State and Private	Indefinite	Indefinite
3. Barbara Crane – Southern Regional Office	Indefinite	Indefinite

Group C

Specialist - 7 representatives (indefinite term)	Terms	
1. Gary Peter – Biotechnology	Indefinite	Indefinite
2. Gary Hodge – Gene Conservation/Racial Variation	Indefinite	Indefinite
3. Alex Mangini – Entomology	Indefinite	Indefinite
4. Jason Smith – Pathology	Indefinite	Indefinite
5. Tom Byram – Seed Orchard and Pest Mangement	Indefinite	Indefinite
6. Tom Fox – Silviculture	Indefinite	Indefinite
7. Jeff Wright – Hardwoods	Indefinite	Indefinite

Group D

Tree Improvements Cooperatives - 3 representatives (indefinite term)	Terms	
1. Tom Byram – Western Gulf Forest Tree Improvement Coop	Indefinite	Indefinite
2. Steve McKeand – N.C. State University Tree Improvement Coop	Indefinite	Indefinite
3. Greg Powell – University of Florida Tree Improvement Coop	Indefinite	Indefinite

Group E

Executive Committee - 4 representatives (2-year terms, Sec/Treas = indefinite)	Terms	
1. C. Dana Nelson – Chair	2011	2013
2. Cetin Yuceer – Vice-Chair	2011	2013
3. Greg Powell – Past Chair	2011	2013
4. Fred Raley – Secretary/Treasurer	Indefinite	Indefinite