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***Populus* Hybridization for the Renewable Transportation Fuels Industry: Integration of Genomic Tools into a Varietal Development Program**

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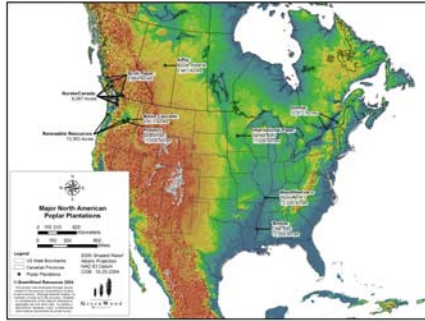
International Poplar
Commission

23rd Session

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Hybrid Poplar in the Pacific Northwest: Biomass Production



1. 13,000 hectares

2. *P. ×generosa*, *P. ×canadensis*

3. Eight year pulp wood rotations at 1,540 trees per hectare

4. Biomass yield up to 18 MT ha⁻¹ yr⁻¹



Hybrid Poplar in the Pacific Northwest: Management for Multiple Markets



Pulp and Paper



Environmental Services



Sawn Wood Products



Cellulosic Ethanol

- **1980s - Pulp and paper industry.**
- **1990s – Environmental services.**
- **2000s - Sawn wood markets.**
- **Future - Cellulosic ethanol industry**

Poplar Biomass for Ethanol Production



- Pacific Ethanol Cellulosic Facility
- 115 million liters per year
- 50 % of feedstock from hybrid poplar
- 400 – 700 metric biomass tons per day

- Greenwood Resources Poplar Farm
- Two year coppice rotation
- Harvest rate of 12 to 20 hectares per day



Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply

April 2005



The Billion Ton Study

The United States National Goal:

1. Displacement of 30% of the country's present petroleum consumption with a sustainable supply of biomass.
2. Requirement of one billion tons of biomass per year.
3. 38% from dedicated perennial energy crops grown on 22 million hectares.
 - *Panicum virgatum*,
 - *Miscanthus x giganteus*
 - *Populus*, *Salix*

Hybrid Poplar's National Strategic Position

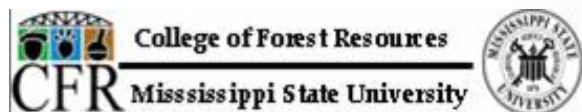


1. **Proven production systems**
 - **Elite varieties**
 - **Wood handling/storage**
2. **Superior environmental benefits**
 - **Lower energy inputs**
 - **Reduced CO₂ / NO₂ emissions**
3. **Excellent opportunity for improvement in biomass yield and quality**
 - **Conventional approaches**
 - **Novel approaches**

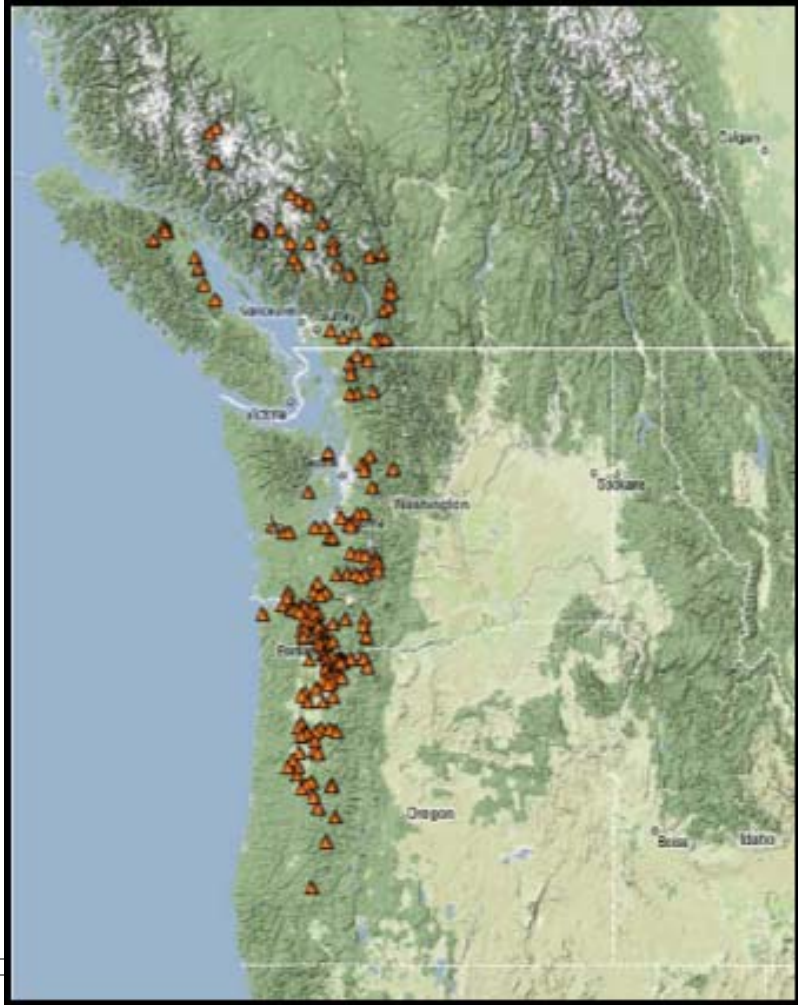
Conventional Approach: The SunGrant Initiative



- National program to coordinate/integrate *Populus* breeding and varietal development
- Goal is elite germplasm of regionally-adapted hybrid taxa for renewable bio-energy feedstock industry.
- Focus on:
 - North Central - *Populus* × *canadensis*,
P. nigra × *P. maximowiczii*
 - Pacific Northwest - *P.* × *generosa*, *P.* × *canadensis*
 - Southeast - *P. deltoides*.



Novel Approach: Association Genetics

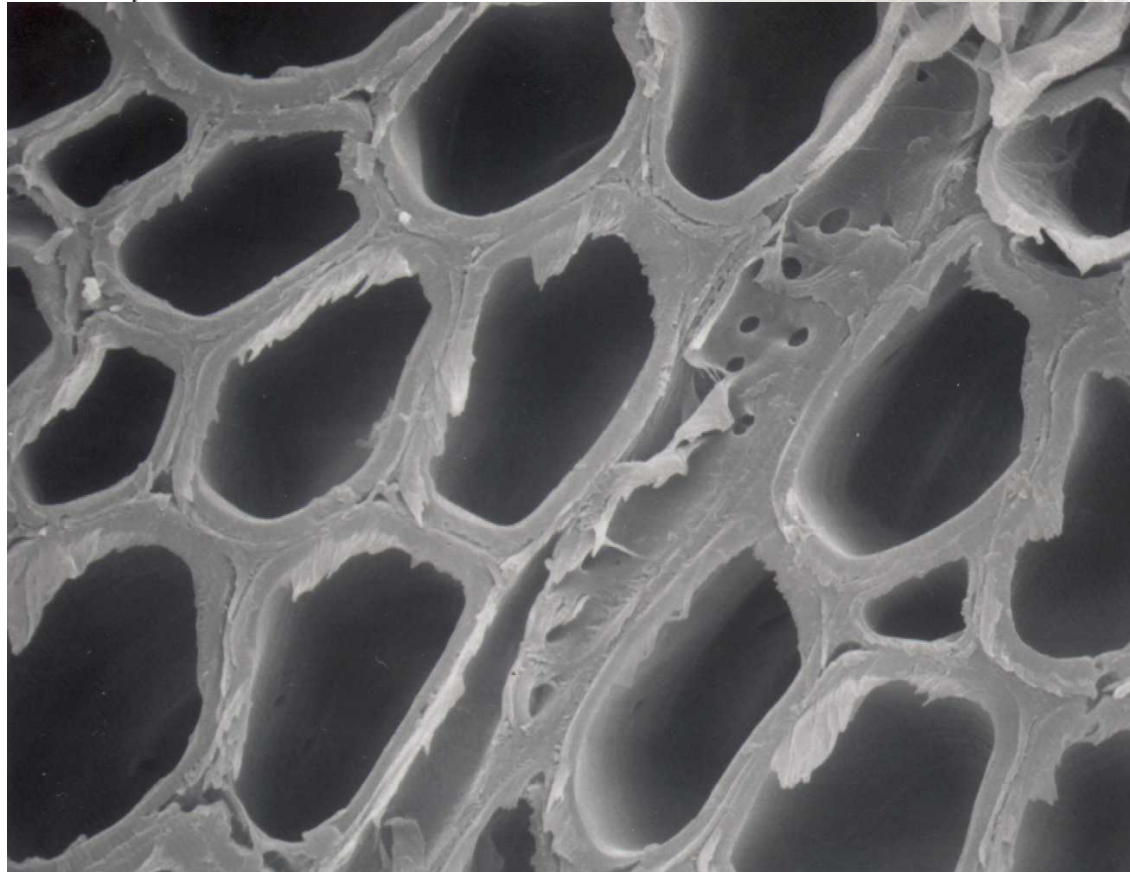


- Goal is the identification of markers associated with genes involved in lignin bio-synthetic pathway
- Two studies of the association between molecular markers (single nucleotide polymorphisms) and biomass compositional traits in *Populus trichocarpa*
- Bioenergy Science Center Oak Ridge National Lab, West Virginia University



- University of California, Greenwood Resources, Michigan Tech, National Renewable Energy Lab





1. Phenotyped a replicated collection of 456 clones for lignin and cellulose composition.
2. Genotyped SNP sequence variation in 40 candidate genes believed associated with lignin and cellulose
3. Presently establishing statistical associations in SNP variation with variation in lignin and carbohydrate chemistry.

Association Genetics of *P. trichocarpa*

Syringyl-to-Guaiacyl Lignin Ratio



Statistic	S/G Ratio
Number of Clones	456
Mean	1.70
Minimum	1.35
Maximum	2.12
Range (% of Mean)	47.80
Phenotypic Standard Deviation	0.16
Clonal Repeatability	0.76



SNP Marker – Trait Associations

The Syringyl-to-Guaiacyl Lignin Ratio

Gene	SNP	R ²	Gene	SNP	R ²
4CL1	06-252	0.0310	F5H1	01-653	0.0262
C4H1	04-219	0.0498	LAC1a	03-98	0.0354
C4H2	09-135	0.0270		05-218	0.0386
	12-151	0.0280		11-493	0.0372
CESA1A	12-223	0.0218		11-70	0.0334
	12-40	0.0218	LAC90a	07-72	0.0333
	16-100	0.0218	PAL2	01-116	0.0584
	16-280	0.0218		01-33	0.0300
	16-506	0.0218	SHMT1	08-428	0.0271
	20-226	0.0218	SUSY1	02-108	0.0214
	16-193	0.0218	gdcH1	05-382	0.0235

Closing

Integration of Novel and Conventional Approaches



A U. S. Initiative in Translational Genomics

1. A national program of government, university, and private companies currently being organized.
2. Adaptation of information derived from genome technologies to applied *Populus* improvement programs.
3. Extend SNP markers developed in *P. trichocarpa* to other *Populus* species.
4. Molecular marker-assisted selection (MAS) to increase the selection efficiency of conventional improvement approaches.

