Genetic Variation and Genomic Associations of Leaf Physiological Traits in an Association Population of *Populus trichocarpa*

Kori K. Ault[1], Steven H. Strauss[1], Anna C. Magnuson[1], Scott Kiel[2], H. Rose McClung[2], Hari Chhetri[3], Upendra Kumar Devisetty[1], Luke Evans[3], Gerald A. Tuskan[4], Wellington Muchero[4], Alejandro Riveros Walker[3], Eli Rodgers-Melnick[5], Stephen DiFazio[3] and Todd N. Rosenstiel[2]

[1]Oregon State University, Corvallis, OR, [2]Portland State University, Portland, OR, [3]West Virginia University, Morgantown, WV, [4]Oak Ridge National Laboratory, Oak Ridge, TN, [5]Cornell University, Ithaca, NY

Summary

We characterized the extent of genetic variation of a number of leaf physiological traits in an association population planted in Corvallis, Oregon. The population contains vegetative propagules from 1,035 genotypes collected in the Pacific Northwest and planted in a randomized block design under the auspices of the DOE Bioenergy Science Center. The population and associated genome sequence data have been shown to be effective for identifying the genomic locations of genes affecting a diversity of adaptive and productivity traits. The traits we studied include dark-adapted fluorescence, chlorophyll content, and height as a measure of biomass productivity. We report on the heritability, genetic correlations, and genomic associations of some of these traits as measured in 2015. All traits were significantly heritable, but the fluorescence-related traits showed very low heritabilities, a likely result of high sensitivity to environment. Several genes were statistically associated with growth and physiological traits.

The association population of *P. trichocarpa* in Corvallis, OR was established from wild collections in 2009, and coppiced and singled in 2013 and 2015. Pictured are A) The association population in August 2015; B-C) Students measuring chlorophyll content (SPAD); and D) Researchers measuring fluorescence.

Methods

Characters measured on each tree:

Height: Measured prior to 2015 growing season using an extendable height pole.

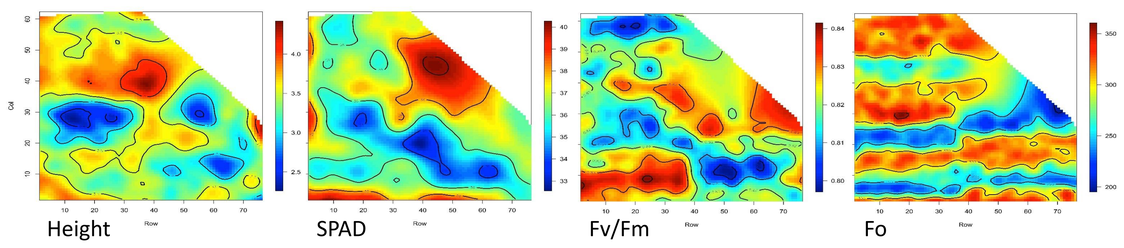
Chlorophyll Content (SPAD): Average of 4 readings per leaf taken on the same leaf used to measure fluorescence. Measurements were taken in Aug 2015 using a SPAD 502DL Plus Chlorophyll Meter (Spectrum Technologies, Inc).

Fluorescence: Leaves were dark-adapted overnight using a specialized cuvette, and measured the following morning. Leaves chosen for measurement were south-facing, fully mature, and representative of the tree. Readings were taken in Aug 2015 using an OS5p+ Pulse Modulated Chlorophyll Fluorometer (OPTI-SCIENCES, Inc). Instrument output includes the fluorescence metrics:

Fo: Minimal fluorescence **Fm:** Maximum fluorescence **Fv:** Difference between Fo and Fm

Analysis: Phenotypic data was corrected for spatial variation using the thin plate spline approach. Residuals from this model were normalized to the phenotypic mean, and genetic effects were estimated using a mixed model with genotype as a random effect. Broad-sense heritability was calculated using variance components from this model. Genome-wide association analysis was conducted using 5,939,334 SNPs derived from whole-genome resequencing data (Evans et al. 2014, Nat. Genet. 46: 1089), and associations were determined using a mixed model analysis with *emmax*, with a kinship matrix and the first two principal components from population structure analysis as covariates.

Thin Plate Spline (TPS) Surface Plots



TPS surface plots showing the spatial distribution of the phenotypic values for height, SPAD, Fv/Fm and Fo. Red indicates higher values and blue indicates lower values.

Graphical interpretation of the TPS plots suggests local-scale environmental influence on phenotypic characters. As such, these TPS corrections have been applied to remove environmental variation from further statistical analyses. The key fluorescence parameter Fo (minimal chlorophyll fluorescence) displays pronounced and regular environmental structuring that appears to be related to rows and/or timing of measurement.

Heritability Estimation (H^2)

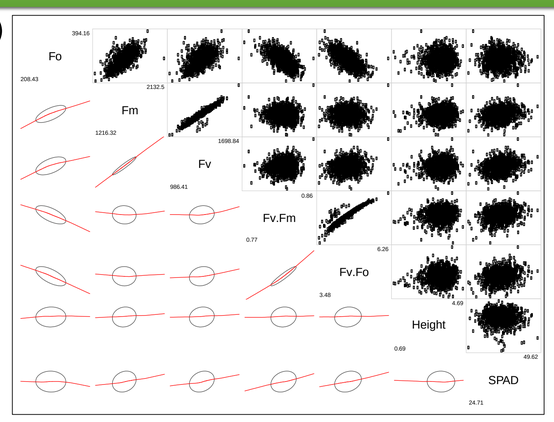
	Heritability (H^2)		
	TPS correction	blk, row, col in model	Fo as a covariate
SPAD	0.355	0.365	–
Height	0.409	0.355	–
Fv/Fo	0.058	0.051	0.037
Fv/Fm	0.057	0.049	0.026
Fm	0.064	0.051	0.019
Fv	0.053	0.039	0.018
Fo	0.105	0.089	–

The Thin Plate Spline (TPS) correction appears to provide the better broad-sense heritability estimates in most cases. Heritabilities (H^2) with TPS correction are all significant ($p < 0.05$) based on a likelihood ratio test, although the H^2 values for the fluorescence parameters are low. Green shading indicates higher heritability values, whereas the red shading indicates lower values.

Results from these preliminary analyses suggest that both tree height and leaf chlorophyll content (SPAD) display moderate broad sense heritabilities. In contrast, F_O displayed an H^2 of only 0.105, suggesting that expression of this key leaf fluorescence metric may be weakly influenced by genotype and strongly affected by environment (see TPS discussion above).

Genetic Correlations

A)



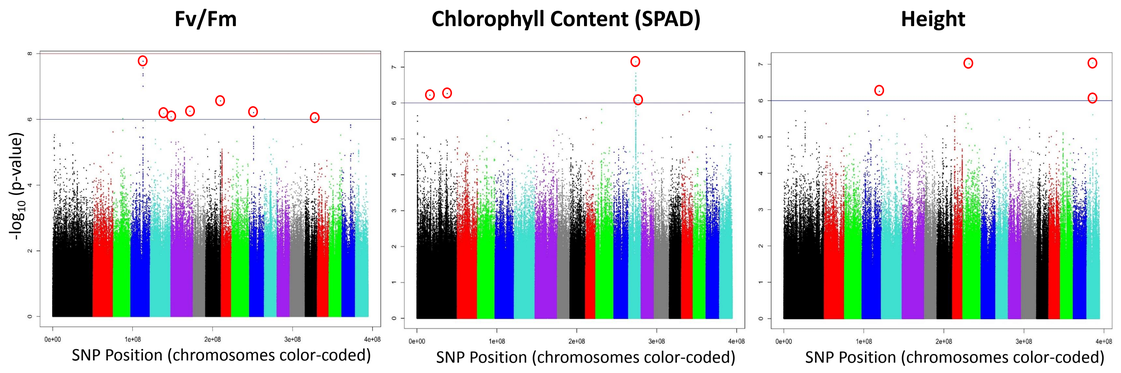
B)

	Fo	Fm	Fv	Fv/Fm	Fv/Fo	Height	SPAD
Fo	1.000	0.668	0.537	-0.636	-0.647	0.035	-0.022
Fm	0.000	1.000	0.956	-0.041	-0.011	0.103	0.196
Fv	0.000	0.000	1.000	0.124	0.162	0.101	0.233
Fv/Fm	0.000	0.176	0.000	1.000	0.939	0.089	0.260
Fv/Fo	0.000	0.717	0.000	0.000	1.000	0.089	0.240
Height	0.245	0.001	0.001	0.003	0.003	1.000	-0.014
SPAD	0.469	0.000	0.000	0.000	0.000	0.645	1.000

A) Correlations are based on the the TPS-adjusted genotypic values. The values in the plot indicate the highest and lowest TPS-corrected mean values for each of the traits. For example, for Fo, 394.16 is the highest TPS adjusted phenotypic value and 208.43 is the lowest value. B) Green indicates higher values and red indicates lower values. Values below the diagonal are p-values and the values above the diagonal are the corresponding correlations (r values).

- All of the fluorescence traits are strongly inter-correlated, as expected based on their physiological bases.
- SPAD was positively correlated with both F_v and Fm, but not with Fo, suggesting that maximal fluorescence is partly a function of chlorophyll content.
- Fluorescence traits were weakly but positively correlated with height, suggesting that these traits have consequences for productivity.

Association Mapping



Genome-wide association analysis based on 5,939,334 (~6 million) SNPs using *emmax*. The SNPs above the blue line in the figure are suggestive associations for Fv/Fm, SPAD, and height ($P<1x10^{-6}$). Information about the gene models closest to these SNPs are in the gene annotation table below.

Gene Models

Trait	SNP position	p-value	Genic Location	Function	Expression
Fv/Fm	Chr03	9.58E-07	Intergenic	Unknown	Highly expressed in root, also in stem nodes, internodes, immature leaves
	Chr04	1.72E-08	Intergenic	Similar to DNA (cytosine-5)-methyltransferase	Highly expressed in root tip, also in young leaves, stem nodes, internodes
	Chr05	6.67E-07	Intergenic	Similar to DNA binding protein-related	Highly expressed in root tip, also in young leaves, stem nodes, internodes
	Chr06	5.69E-07	Intergenic	Unknown, similar to Arabidopsis expressed protein	Highly expressed in stem internodes, also stem nodes and leaves
	Chr06	8.42E-07	Intergenic	Copper transport protein ATOX1-related (PTHR22814)	Expressed in stem and leaves
	Chr08	2.76E-07	Intergenic	Stigma specific protein, Stig1	Highly expressed in root tip, also young leaves, stem nodes, internodes
	Chr11	6.24E-07	Genic	Unknown	Highly expressed in root and stem nodes, also in young leaves, floral buds
			Intergenic	Unknown	Unknown
	Chr12	9.73E-07	Intergenic	Homolog Arabidopsis Ubiquitin-conjugating enzyme	Unknown
	Chr15	9.25E-07	Intergenic	Unknown	Expressed in stem and leaf, low expression in root
SPAD	Chr01	5.47E-07	Intergenic	Cytochrome P450 CYP2 subfamily protein	Expressed in stem nodes and internodes
	Chr01	6.11E-07	Intergenic	Unknown	Unknown
	Chr12	7.09E-08	Intergenic	Predicted E3 ubiquitin ligase	Highly expressed in root tip, female floral bud, stem nodes, internodes
	Chr12	8.86E-08	Intergenic	Unknown	Highly expressed in root
	Chr12	8.54E-07	Intergenic	Similar to glyceraldehyde-3-phosphate dehydrogenase	Highly expressed in immature leaf, low in stem nodes and internodes
Height	Chr04	5.60E-07	Intergenic	Similar to auxin response factor 10	Highly expressed in root, low expression in stem internode
	Chr10	9.75E-08	Intergenic	Cellulase (glycosyl hydrolase family 5)	Low expression in root
	Chr19	9.14E-08	Intergenic	Unknown	Unknown
	Chr19	8.54E-07	Intergenic		

Conclusions

Heritabilities and genetic correlations suggest statistically significant but weak genetic control and physiological associations among traits. It appears unlikely that these measures will substantially improve upon measures of growth as indices of heterosis and stress tolerance.

We examined the large-scale genetic associations of leaf chlorophyll fluorescence, in association with other metrics, in *Populus* trees. Results from initial gene association studies suggest several key genes, especially those involved in cellular redox status (e.g. ATOX1, Cytochrome P450 CYP2, glyceraldehyde 3-P dehydrogenase) may be involved in influencing *Populus* leaf chlorophyll fluorescence and chlorophyll content. Further association studies and experimental validations are needed to test these correlations.

We will next evaluate the relative importance of structural polymorphisms in determining adaptive trait variation in *Populus*, focusing in particular on the roles of interspecific SNP and insertion/deletion polymorphisms in determination of heterosis.

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