Genetics of Adaptation and Stress Mitigation Traits in a Clonal Loblolly Pine (*Pinus taeda* L.) Association Mapping Population

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Introduction

We are searching for alleles that are associated with environmental adaptation and stress mitigation traits in loblolly pine, and would like to develop guidelines for selecting loblolly pine trees that would be more resilient to altered climatic conditions. Single nucleotide polymorphism (SNP) discovery is being conducted using a target sequence capture method. This summer, we will utilize the PINEMAP undergraduate fellowship program to collect and analyze phenotypic data at one field site in southeast Mississippi. These SNP genotypes and field phenotypes will be used in association genetic analyses to discover alleles affecting the measured traits.

Objectives

1. To measure and conduct mixed model analyses on the adaptation, mitigation and productivity traits in the clonal ADEPT2 population trees.
2. To associate these traits with exome- based SNPs.

Figure 1. ADEPT2 population at the Harrison Experimental Forest

Material and Methods

Rooted cuttings from 384 unrelated trees (i.e., clones) sampled across a natural range of loblolly pine were established at Harrison Experimental Forest at the Southern Institute of Forest Genetics in southeast Mississippi during the spring of 2011 (Fig. 1). A randomized incomplete block alpha lattice design (r=3, s=24, k=16) was used, with 3 replications of 24 incomplete blocks of size 16 (4 trees x 4 trees). Clones were represented as single tree (i.e., ramet) plots.

The following traits will be measured: 1) Specific leaf area: Ten fresh bundles from each tree will be collected. LAI 3000 will be applied to measure the area of a subset of needles. After the needles are dried in the oven, the dry weight will be measured. SLA is the ratio of leaf area to leaf dry mass. 2) Stomata density: Four fresh bundles from each of three heights (top, middle and bottom) of each tree will be collected. The number of stomata per unit area will be calculated using a digital microscope camera. 3) Wood specific gravity: A wood core from each tree will be collected by an increment borer. The maximum moisture content technique will be applied to calculate the values. 4) Branch angle: The branch angle relative to vertical of three branches near breast height of each tree will be measured using a digital level. 5) Carbon isotope discrimination and leaf nitrogen: Needle samples from each tree will be dried and grounded to fine powder. Then the samples will be sent to the Stable Isotopes for Biosphere Science Laboratory at Texas A&M University for analysis.

A mixed model analysis will be used to assess the clonal effects for the measured traits using the following model:

$$y_{ijk} = \mu + r_i + b_k(r_i) + c_j + r_i c_j + e_{ijk}$$

y_{ijk} is the phenotypic value for the jth clone in the ith replication and kth block. μ is population mean, r_i is the fixed variable of replication (i=1-3), c_j is the random variable of clone (j=1-384, approx.NID(0, σ^2_c)), $b_{k(ri)}$ is the random variable of block nested within replication (k=1-24, approx. NID(0, $\sigma^2_{b(r)}$)), $r_i c_j$ is the random variable for the interaction of replication by clone (approx.NID(0, σ^2_{rc})), and e_{ijk} is the error term (approx.NID(0, σ^2_e)).

SNP discovery and genotyping are being conducted using Agilent SureSelect Target Enrichment and the Nimblegen SeqCap EZ System .

Preliminary Results

1. A SNP detection pipeline was developed using the loblolly pine exome and the Agilent SureSelect Target Enrichment method. Under the filtering condition of minimum read depth=10, at least 30% of total uniquely mapped reads contain an alternate allele, 2,810,893 SNPs were detected by SAMtools. Genotyping using Nimblegen SeqCap EZ System with the exon annotation based probes is underway.
2. Probes were designed based on 51Mb exon regions of the loblolly pine v.1.01 reference genome (PINEREFSEQ project, http://loblolly.ucdavis.edu/bipod/ftp/Genome_Data/genome/pinerefseq/Pita/v1.01/). The designed probes covered 90.2% of the exon regions. These probes will be utilized in the exome capture experiment.
3. Multiplexing strategies worked well for capturing targeted sequences and SNP discovery. A higher multiplexing level reduces the coverage of each sample, but still provides high numbers of SNPs for efficient genotyping.
4. Preliminary analysis shows there is spatial variability in the ADEPT2 population. A spatial covariance structure constructed by spherical method could fit the data better.

Next step

Finish genotyping and phenotyping for 384 trees in the ADEPT2 population, then associate the SNPs with traits listed here. We hope to discover molecular markers which can help to better screen the genotypes of loblolly pines under changing climate.

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USDA United States Department of Agriculture National Institute of Food and Agriculture