Enhanced Resistance Pines for Improved Renewable Biofuel and Chemical Production

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Project Goals: Our goal is to genetically increase constitutive terpene defenses of loblolly and slash pine to enhance protection against pests and pathogens and at the same time expand terpene supplies for renewable biofuels and chemicals.

Abstract: Today, the southeastern U.S. hosts the world's largest biomass supply chain, annually delivering 17% of global wood products, more than any other country. This well-developed regional supply chain supports southern pine genetic improvement, seedling production and planting, silviculture, harvesting, and transportation annually delivering ~250 million tons of pine wood to integrated manufacturing facilities. The SE US also houses the U.S. pine chemicals industry, which is the oldest and one of the largest US renewable hydrocarbon chemical industries. Our focus is to increase constitutive terpene production to enhance loblolly and slash pine resistance to pests and pathogens. Enhanced genetic resistance in these commercial pine species is critical to protect against widespread losses as biotic pressures increase due to global warming, land-use change, and introduced exotic organisms.

Increasing pine terpenes is also well aligned with the needs of the developing bioeconomy. Today, commercial scale collection of pine terpenes occurs from live trees by tapping, from stumps by solvent-based steam extraction, and from pulp mills as co-products. US pulp mills recover ~900,000 tonnes y^{-1} of terpenes and fatty acids supporting specialty chemical biorefineries that compete in markets with petroleum-derived feedstocks, supporting our concept that biofuels from pine terpenes could be profitable without subsidy if supply was increased. Proven technologies exist to convert efficiently pine terpenes to biofuels: a 30 million gallon y^{-1} bio-refinery produces renewable diesel from pine terpenes and fatty acids, and pine monoterpenes can be efficiently dimerized to produce a replacement for JP10, the highest density jet fuel. Higher wood terpene content will increase the yield of bioenergy per unit mass in the rapidly developing wood pellet industry, as pine terpene supply is currently limited by relatively low average wood terpene content.

Pine terpenes evolved as a primary chemical and physical defense system and are a main component of a durable, quantitative defense mechanism against pests and pathogens. In previous research we demonstrated that terpene defense traits are under genetic control and behave as quantitative traits, and have used genetic engineering to validate 12 genes that can significantly increase wood terpene content.

In objective one, we are integrating existing and new genome wide association (GWAS) genetic results with RNA expression, QTL mapping, and allele frequency information in known high oleoresin flow selections and our breeding populations to discover and validate loblolly and slash pine alleles/genes that are important for resistance. GWAS analyses of constitutive oleoresin flow, wood mono- and diterpene content and resin canal number with 82,000 biallelic SNPs were completed for our CCLONES population and constitutive oleoresin flow, mono- and diterpene

content are complete and resin canal number is in progress for our ADEPT 2 population. In the ADEPT2 population, we also measured induced oleoresin flow after treating clones with methyl jasmonate (MeJA). While our goal is to increase constitutive terpene defenses, we use MeJA to induce defense responses to identify the genes and genetic architecture of resinosis. Quantitative genetic and association analyses are in progress with ~2.2 million biallelic SNP markers. In our pseudo-backcross population between one F1 slash x loblolly hybrid genotype backcrossed to slash and loblolly genotypes, we collected constitutive oleoresin flow for future QTL mapping. We measured constitutive and induced resin flow in mature USFS known high-yielding slash pine selections in two seed orchards. We tapped 124 ramets from 31 clones in FL and 55 ramets from 14 clones in GA. Analysis to further characterize these high-yielding genotypes is underway.

We conducted a time course experiment to identify early, mid and late genes expressed in differentiating resin ducts. In addition to available cell specific RNAseq data, we also created 88 new RNAseq libraries from cambial zone tissue collected from days 0, 1-14, 17, and 21 after treatment with MeJA, an inducer of new axial resin canals. We also constructed 43 libraries from transgenic pines expressing pine genes with significantly greater wood terpene content. Anticipating integrating these datasets, we created a database of all genomic resources including porting pine ref v1.01 to which most SNPs were designed to v2.01. We created a more complete transcriptome with 64,671 genes, including existing contigs, PacBio reads and pine ref v2.01 predicted transcripts. This improved transcriptome has 80% full-length transcripts for mapping RNAseq reads for differential expression and functional annotation of significant SNP markers.

In objective two, we are using information from objective one to accelerate breeding for increased resistance in loblolly and slash pine through marker assisted introgression, and will develop and test genomic selection models to accelerate breeding of resistant slash pine.