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Final Report for "The Genomic Basis of Heterosis in High-Yielding Triploid Hybrids of Willow (*Salix* spp.) Bioenergy Crops"

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Collaborating entities: Steve DiFazio, West Virginia University; US DOE Joint Genome Institute; HudsonAlpha Institute for Biotechnology

Abstract (5000 char):

Many studies have highlighted the complex, multigenic basis for heterosis (hybrid vigor) in inbred crops. Despite the lack a consensus model, it is vital that we turn our attention to understanding heterosis in undomesticated, outcrossing, heterozygous, and often polyploid species, such as willow (*Salix*). Shrub willow is a dedicated energy crop and is bred to be fast-growing and high-yielding on marginal land without competing with food crops. A trend in willow breeding is the consistent pattern of heterosis in triploid progeny produced from crosses between diploid and tetraploid species. Critical in understanding heterosis, the heritability of gene expression is dependent on allele-specific expression by local and remote factors in the genome. Here, we tested whether differentially expressed genes are responsible for heterosis in triploid crosses made between diploid *S. purpurea*, diploid *S. viminalis*, and tetraploid *S. miyabeana* parents. Three biological replicates of progeny and parent shoot tips were collected after 11 weeks in the greenhouse and individually sequenced via RNA-Seq (2×101). Our results highlight regulatory factors influencing differential expression and top modules of co-expressed genes correlated with heterosis for phenotypes collected in the greenhouse and in the field. We also show in diploid F_1 and F_2 *S. purpurea* that expression-level dominance and sex dimorphic expression is pervasive in shoot tips. Gene expression in triploids largely matches expected dosage values, but there are interesting categories of genes whose expression is divergent from expected dosage of alleles. Candidate genes have been identified whose differential expression be confirmed via allele-specific assays. Altogether, these data will be used to develop predictive models of heterosis and complement the growing genomic resources available for the improvement of shrub willow bioenergy crops.

Keywords: heterosis; willow; hybrids; gene expression; genomics; triploid; yield

Background: Yield improvement in many crops has been based on capturing heterosis, but even in well-studied species the complex genetic basis for hybrid vigor is poorly understood. Breeding for yield improvement in willow bioenergy crops has relied primarily on capturing hybrid vigor through interspecific hybridization, yet we know little about the genomic basis for heterosis in these hybrids, the best of which are triploids resulting from crosses of tetraploids with diploids. In this project, we are asking how the gene expression patterns in willow hybrids are related to their yield potential and other traits important for biofuels production. In particular, we will test if there is a bias in the expression of key genes from one parent versus the other in species hybrids, and whether there is a gene dosage effect skewing gene expression patterns in triploid progeny compared with their diploid and tetraploid parents.

Project Results for Planned Tasks:

Objective 1. Quantify heterosis for yield and biomass traits across eight families representing intraspecific diploids and interspecific diploid and triploid progeny.

Task 1.1: Development of diploid and triploid* Salix *pedigrees. The original scheme of the project was based on eight families to compare reciprocal crosses of diploid hybrids and triploid hybrids. Seven of those eight families were produced, but one triploid family with a tetraploid mother and diploid father could not be recovered. Most families have more than the target number of progeny (100), while two have less than 100 progeny (Fig. 1, Table 1). One family only had 68 progeny in the trial and could not be further scaled up, because the female was heavily damaged by potato leafhopper and did not flower. The other produced only 25 viable progeny for the trial, as the female was very recalcitrant to controlled pollination in the greenhouse. Seedlings of all the families produced were established in nursery beds by the end of 2013 to provide an archival source of propagation material.

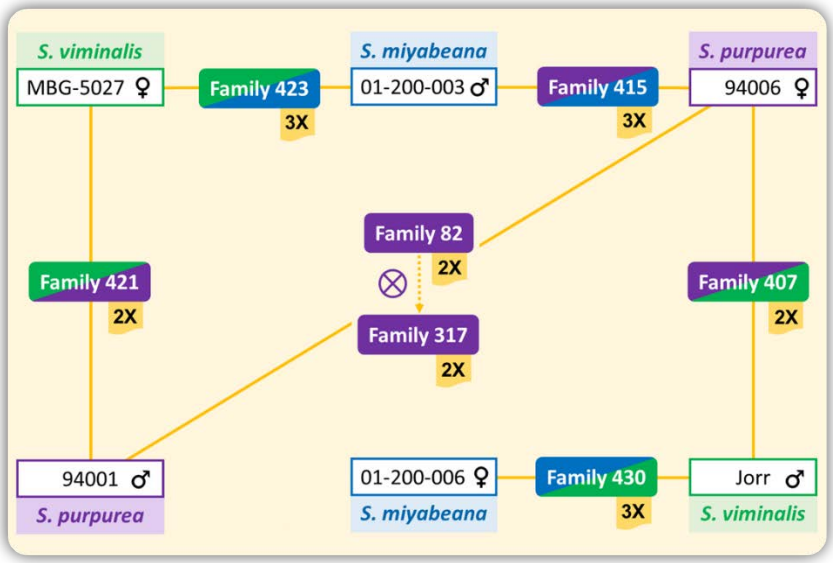

Fig. 1. Pedigree scheme of intraspecific diploid, interspecific diploid, and interspecific triploid families.

Table 1. Detailed pedigree and number of progeny for families described above.

Pedigree (ID)	Female parent [ploidy]	Male parent [ploidy]	Family ploidy	Total progeny	Progeny in trial
SpuF_1 (082)	*S. pur* 94006 [2X]	*S. pur* 94001 [2X]	**2X**	**425**	**100**
SpuF_2 (317)	*S. pur* 9882-41 [2X]	*S. pur* 9882-34 [2X]	**2X**	**495**	**486**
Spu x *Svi* (407)	*S. pur* 94006 [2X]	*S. vim* 'Jorr' [2X]	**2X**	**290**	**100**
Svi x *Spu* (421)	*S. vim* MBG5027 [2X]	*S. pur* 94001 [2X]	**2X**	**250**	**100**
Spu x *Smi* (415)	*S. pur* 94006 [2X]	*S. miya* 01-200-003 [4X]	**3X**	**306**	**100**
Svi x *Smi* (423)	*S. vim* MBG5027 [2X]	*S. miya* 01-200-003 [4X]	**3X**	**75**	**68**
Smi x *Svi* (430)	*S. miya* 01-200-006 [4X]	*S. vim* 'Jorr' [2X]	**3X**	**39**	**25**
Smi x *Smi* (425)	*S. miya* 01-200-006 [4X]	*S. miya* 'SX64' [4X]	**4X**	**163**	**100**

Task 1.2 Establishment of replicated field trials for phenotyping. Cuttings were harvested from the seedling plants in the nursery beds described above and were used to establish a replicated field trial in summer 2014. It was decided not to measure growth of individual seedling plants in the nursery beds, as the unreplicated data would be of little value in representing the growth potential of these progeny. The design of the field trial included up to 100 randomly selected progeny from each family or the maximum number for which cuttings could be collected, as well as the parents of each family and several commercial cultivars as checks. The trial includes all possible individuals of family 317, which is a *Salix purpurea* F_2 population that has been fully genotyped using genotyping-by-sequencing to generate a high-resolution linkage map, which will allow us to readily map traits measured in this family of 485 individuals. Each entry was planted in three-plant plots in single row design for ease of measurement and harvest in each of four completely randomized blocks in a single field. The plants were coppiced after the 2014 season and again after the 2016 season.

Task 1.3 Phenotyping of replicated field trials. Measurements of leaf and stem growth, including stem number, height, stem diameters, relative growth rate, and many leaf traits were collected during and after the 2015, 2016, and 2017 seasons. To determine biomass composition, two TA Instruments Q500 Thermogravimetric Analyzers (TGA) were installed and have been used to optimize and improve upon the method described in Serapiglia et al (2009). A set of 100 willow samples, including diploid, tetraploid, and triploid genotypes, were characterized through wet chemical analysis with complete sugar profiles. Cellulose, hemicellulose, lignin, and ash content were analyzed by HR-TGA and multivariate analyses were performed to optimize the method. This improved method was used to analyze diploid, tetraploid and triploid genotypes over the three growing seasons of a harvest cycle in the replicated 2008 Selection Trial in Geneva. There were significant differences in biomass composition among the 75 genotypes in the trial, including significant differences according to ploidy, with triploids and tetraploids displaying lower lignin than diploids. Also, tetraploids displayed lower specific gravity. There were also differences in S:G lignin ratios among the 75 genotypes examined, in addition to significant correlations with willow growth traits, yield, and composition. These differences suggest that a long-term strategy of breeding for triploid progeny will generate cultivars with improved growth traits and wood composition for conversion to biofuels. Stem samples were collected from every entry in the trial, specific gravity was measured, the stems were milled to 0.5 mm, and analyzed by HR-TGA. QTL for the phenotypic traits that were measured are being mapped for the diploid families that have been genotyped. Individuals with extreme variation in wood composition will be used in a follow-on project to breed for cultivars with unique wood compositional traits.

Objective 2. Determine allele-specific gene expression in intraspecific diploid and interspecific diploid and triploid hybrids.

***Task 2.1. Illumina RNA-Seq of parents and progeny*.** Ten randomly selected progeny from the six families that had been produced by 2012 (SpuF1-082, SpuF2-317, SvixSpu-421, SpuxSvi-407, SpuxSmi-415, and SvixSmi-423, see Table 1) plus all of the parents of those families (94006, 94001, 9882-34, 9882-41, MBG5027, 'Jorr', and 01-200-003) were grown from cuttings in the greenhouse in replication to produce tissue for RNA-Seq analysis. Cuttings were collected from nursery beds in January 2013 and planted in potting mix in 2.5-L pots pots in the greenhouse. A

completely-randomized block design was used where four replicates per genotype were randomized on each of four greenhouse benches within the same house. All plants were adequately watered and fertilized regularly to provide optimum growing conditions. After three months of growth all stems on each plant were measured to calculate sum of stem height per plant and tissue was collected for RNA isolation. The plants became infested with western flower thrips, which caused some damage to leaves and shoot tips. We applied pesticides and used beneficial mites to minimize the damage prior to tissue collection, but some genotypes suffered more damage than others. This damage will be considered as we analyze RNA-Seq results. From each of the four replicates from each genotype, shoot tip tissue was pooled together and young stem internode tissue was pooled. The tissue was flash frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated, checked for quality, cDNA libraries were constructed and subjected to RNA-Seq at JCVI. Young leaf tissue was collected from a single plant of each genotype and sent to Dr. K. Arumuganathan (Aru) at the Benaroya Research Institute for flow cytometric analysis of nuclear DNA content. The results indicate that all of the DNA content values for the progeny are in the expected range for diploids (SpuF1-082, SpuF2-317, SvixSpu-421, SpuxSvi-407) and triploids (SpuxSmi-415 and SvixSmi-423) except for one individual in SpuF2-317 and one in SpuxSvi-407, which had nuclear DNA content readings approaching those of triploids. The flow cytometry was repeated, revealing that the 317 individual was, in fact, diploid, while field surveys suggest that the 407 individual was a rare case of an individual from another family (probably 415, a triploid pedigree) that was mistakenly mixed in with the 407 family in the nursery beds.

A second greenhouse experiment was established using 12 individuals from all seven diploid and triploid families, as well as a tetraploid family. Extensive phenotyping was carried out on these plants during the 12 weeks they grew under ideal conditions in the greenhouse. Shoot tip tissue was collected from the triploid progeny and their parents an used to construct cDNA libraries with three biological replicates of each genotype. These were subjected to RNA-Seq at JCVI.

RNA-Seq data was analyzed using the *Salix purpurea* 94006 ver.1.0 genome sequenced and annotated at US DOE Joint Genome Institute (JGI) with assembly done at JCVI. The RNA-Seq analysis indicated that more genes were expressed in the *S. purpurea* F_1 family, than in the F_2 family. There were even more genes expressed in diploid species hybrids and yet even more in triploid species hybrids. However, the overall expression level in progeny expressed as mean $\log_2$(CPM) was lower than the mid-parent mean of expression levels in the parents. A multi-dimensional scaling (MDS) plot of gene expression indicated that expression patterns largely fall intermediate between parents (Fig. 2), suggesting additive patterns of inheritance. There were also strong patterns of differential expression by tissue type between shoot tips and stem internodes. We also detected strong patterns of gene expression correlated with the sex of the plant, probably because at the time of shoot tip collection, there were genes involved in floral determinacy being expressed. This differential expression by sex was mapped to Chr 15 (Fig. 3), the predicted region of the sex determination region (SDR). There was also increasing numbers of genes showing differential expression between parents in interspecific diploids and triploids.

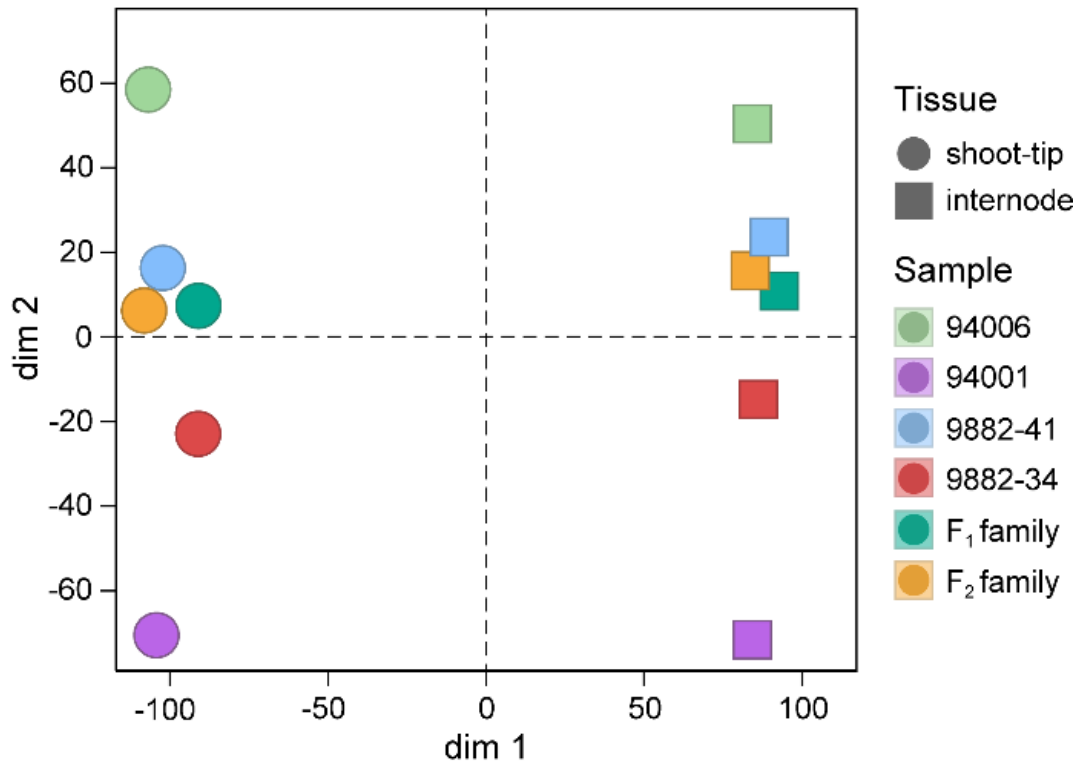

Fig. 2. MDS plot of gene expression in intraspecific diploid families showing largely additive patterns of expression and resolution based on tissue type.

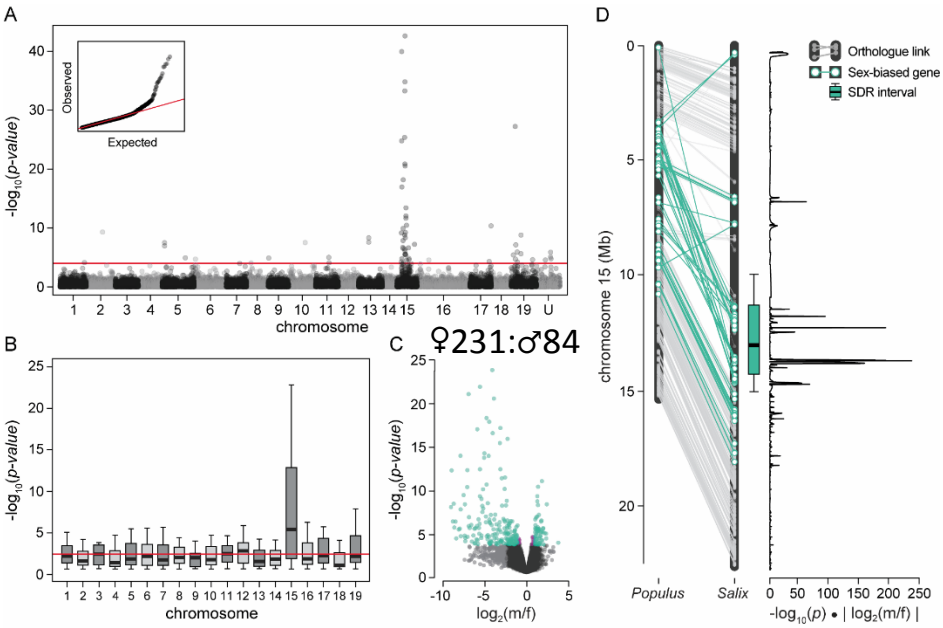

Fig. 3. Differential expression patterns based on plant sex. (A) Mapping to chromosome 15; (B) DE genes by chromosome, (C) abundance of female-specific genes,

Task 2.2. SNP analysis and quantification of allelic expression. We collaborated with JGI to conduct whole-genome shotgun sequencing of a wild diploid, female accession *Salix purpurea*, clone ID 94006. ALLPATHS-LG was used at JCVI to assemble sequences representing ~140X coverage of paired-end sequences and all of the mate-pair libraries (4.5 Kb, 5.3 Kb, 6.5 Kb), producing contigs with L50=46 kb and scaffolds with L50=191 kb. The ALLPATHS-LG assembly has a total size of 348 Mb and a total span of 392 Mb (11% Ns). This assembly is still relatively fragmented due to the high level of heterozygosity (1 SNP per 120 bp, or 0.8%) and low yield of long insert mate pair library. Assessment of the assembly quality against willow BACs and transcripts suggested that ~ 78% to 85% of the willow genome is captured in the current assembly.

We further integrated the ALLPATHS assembly with high-resolution genetic maps developed using genotyping-by-sequencing (GBS) to create a chromosomal assembly (v.1.0) in collaboration with the Steve DiFazio lab at West Virginia University. The scaffolds were anchored to 19 pseudo-chromosomes and ordered using a novel algorithm to maximize co-linearity with all genetic maps while simultaneously minimizing the total genetic distance traversed (Rodgers-Melnick et al., in preparation). A total of 276 Mb (70%) sequences can be anchored onto the chromosomes. Scaffolds with conflicting placement were resolved in favor of the scaffold with the most markers hitting the chromosome if the difference was larger than 3; the scaffolds were labeled as unplaced otherwise. The remaining unplaced scaffolds contain an overall size of 116 Mb (30%). The scaffolds within the pseudomolecules were ordered on the basis of segregation pattern when there are at least 4 GBS markers or through a propagation algorithm using *Populus* alignments as hints on scaffolds with insufficient number of markers. All pseudomolecules are globally oriented with respect to *Populus trichocarpa* chromosomes.

Willow genome annotation was completed in collaboration with JGI. JCVI supplied a set of transcript assemblies, JGI predicted gene structures, JCVI ran its gene function annotation pipeline and assigned scaffold-level identifiers to all genes. The *Salix purpurea* genome and annotation were integrated into Phytozome and released publicly in 2015. In order to provide reference SNP data for all parents used in the various crosses, we sequenced each parent individual using 100 bp paired end reads on ~ 500 bp fragments. The reads were mapped to the reference genome (94006) to produce a set of SNP calls for each parent. These were used to investigate parent of origin/allelic expression in the progeny of each cross.

To study inheritance patterns of gene expression in *Salix* purpurea, we completed RNA-seq of the parents (female 94006 - male 94001) and 10 progeny of the family 10X-082. With two tissues per individual (shoot tip and stem internode), a total of 24 bar-coded libraries were sequenced in four lanes of Illumina HiSeq with single end 100 bp reads. We conducted analysis in two stages: 1) Determine expression levels for all genes in each individual and tissue. Compare with parental values to seek evidence for heterosis; 2) determine the contribution of paternal and maternal alleles to levels of expression.

Expression levels - Reads from each individual and each tissue were mapped to the annotated 94006 reference genome using CLC Bio and RPKM values determined. An index for heterosis was calculated for each gene, individual and tissue as $\log_2$(RPKM_sample/RPKM_parental_mean). Across all individuals and all genes, values for this

ratio ranged from >2^{10} to <2^{-10}. i.e. many genes were expressed 2^5 (32) or more times higher than the parental mean. The number of genes in the entire genome showing significant expression above or below the mean of the two parents varied substantially among the 10 progeny from the cross. We chose loci (SNPs in genes) that were homozygous in each parent, but polymorphic and differentially expressed between parents to study expression inheritance. In *S. purpurea*, there was a high proportion of dominant inheritance, with a skew toward maternal parent dominance. For the interspecific diploids and triploids grown in the first greenhouse trial, the progeny RNA was pooled for RNA-Seq and compared with the parents. The interspecific diploids (*S. purpurea* × *S. viminalis* and *S. viminalis* × *S. purpurea*) showed strong dominance of gene expression, with very little additive expression. This was also true for the interspecific triploids (*S. purpurea* × *S. miyabeana* and *S. viminalis* × *S. miyabeana*), which were strongly biased toward maternal parent (diploid) dominance (Fig. 4). In the second greenhouse experiment, RNA libraries were made for each progeny genotype with biological replication, which resulted in different outcomes in the analysis if allele-specific expression of triploids. Rather than strong maternal inheritance of expression, there was strong dominance by the tetraploid parent, but also a major component of additive expression inheritance (Fig. 5).

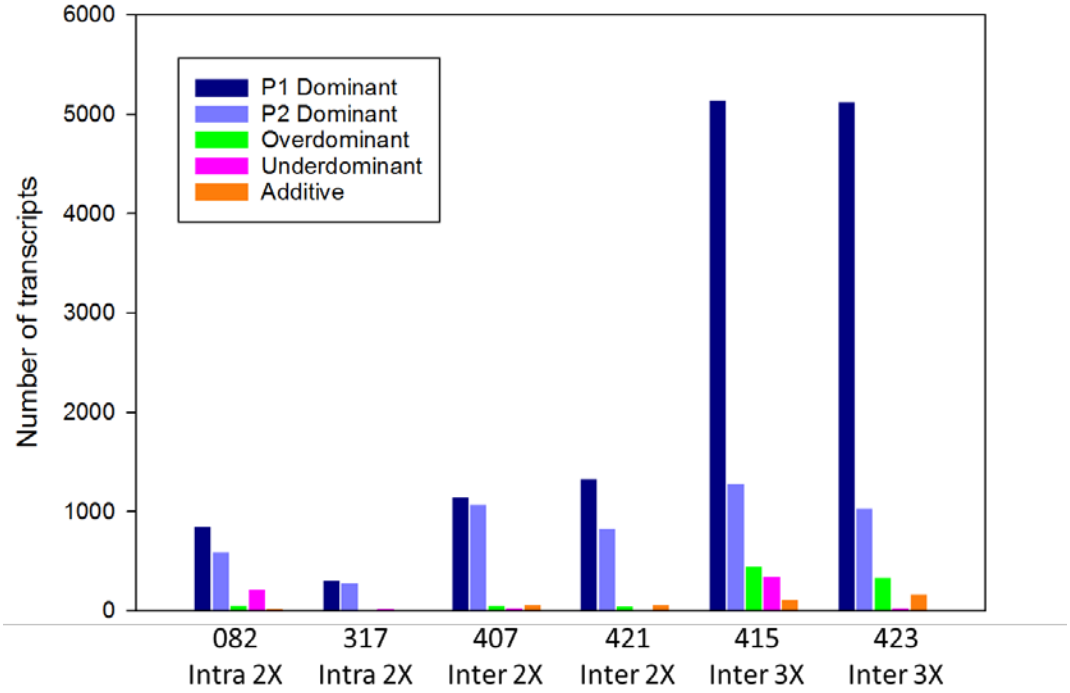

Fig. 4. Strong dominance in expression inheritance, with particular maternal (diploid) dominance in the triploid progeny.

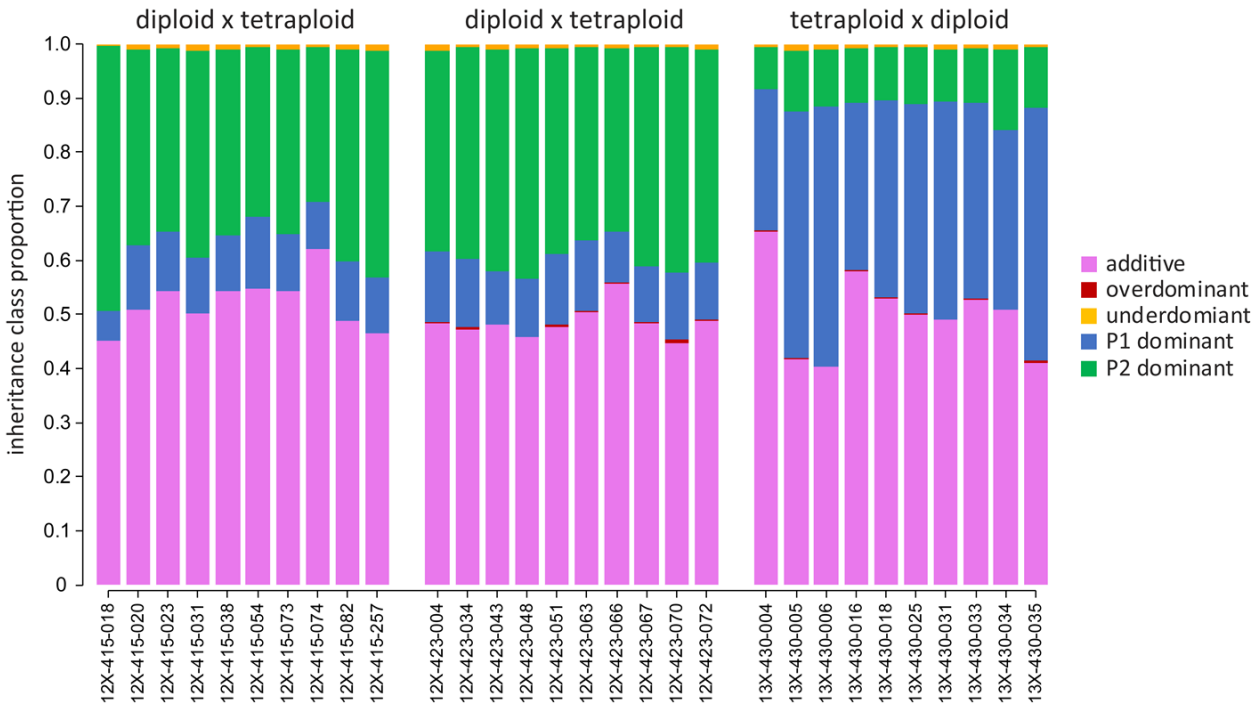

Fig. 5. Strong dominance in expression inheritance from the tetraploid parent, with a high proportion of additive inheritance of expression as well in the triploid progeny.

These data were also used to study the allele-specific regulatory control of genes inherited from either parent. By comparing the expression in progeny with that in parents, we determined if transcriptional activity in the progeny was primarily under *cis* regulation (consistent with expression in the parent) or under *trans* regulation (expression of the two alleles in the progeny is identical). Alternatively, expression can be a combination of *cis* and *trans* or can show novel levels in the progeny due to compensatory action. In *S. purpurea* shoot tips, the differential allele-specific patterns were largely compensatory, followed by *cis* with some *trans* regulation. In stem internodes, the regulation was primarily *cis* followed by *trans*, with little compensatory regulation (Fig. 6). Regulatory divergence in the triploid progeny had a strong component of *cis* regulation as well, followed by *trans* regulation (Fig. 7). These proportions did not vary depending on the ploidy of the parent. While most genes were expressed as predicted based on the relative dosage (1/3 expression of the allele from the diploid parent and 2/3 expression of the allele from the tetraploid parent), there were a number of genes that showed dysregulation based on dosage. Among those genes showing expression levels significantly different from expected dosage were heat shock proteins and proteins involved in electron transport.

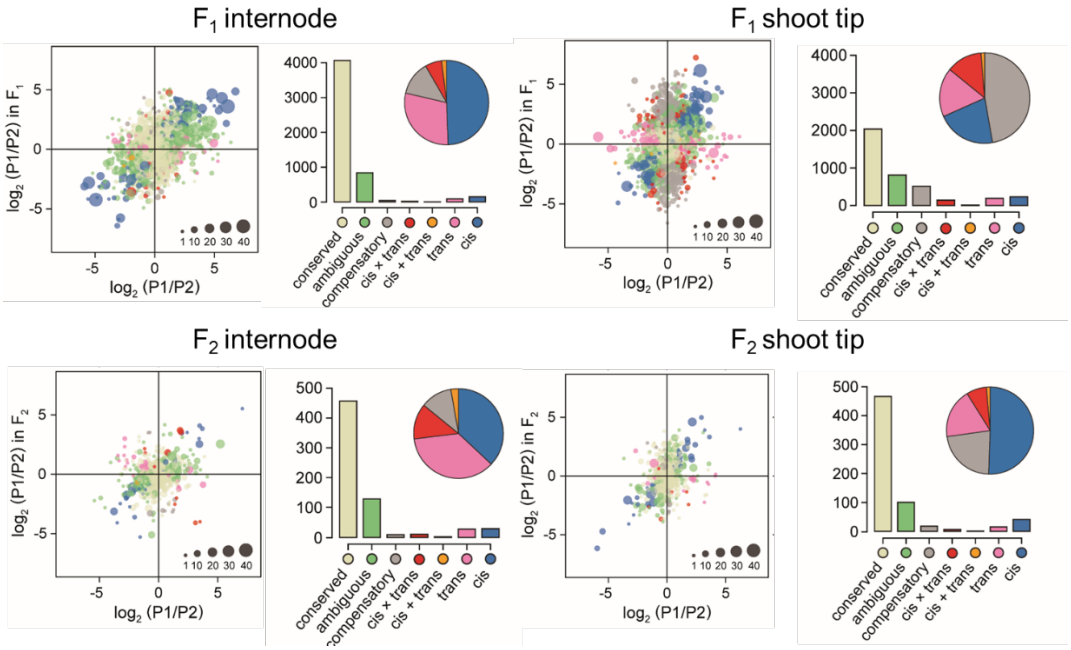

Fig. 6. Patterns of regulatory control of allele-specific expression in *S. purpurea* progeny, with strong compensatory control in shoot tips and cis control in stem internodes.

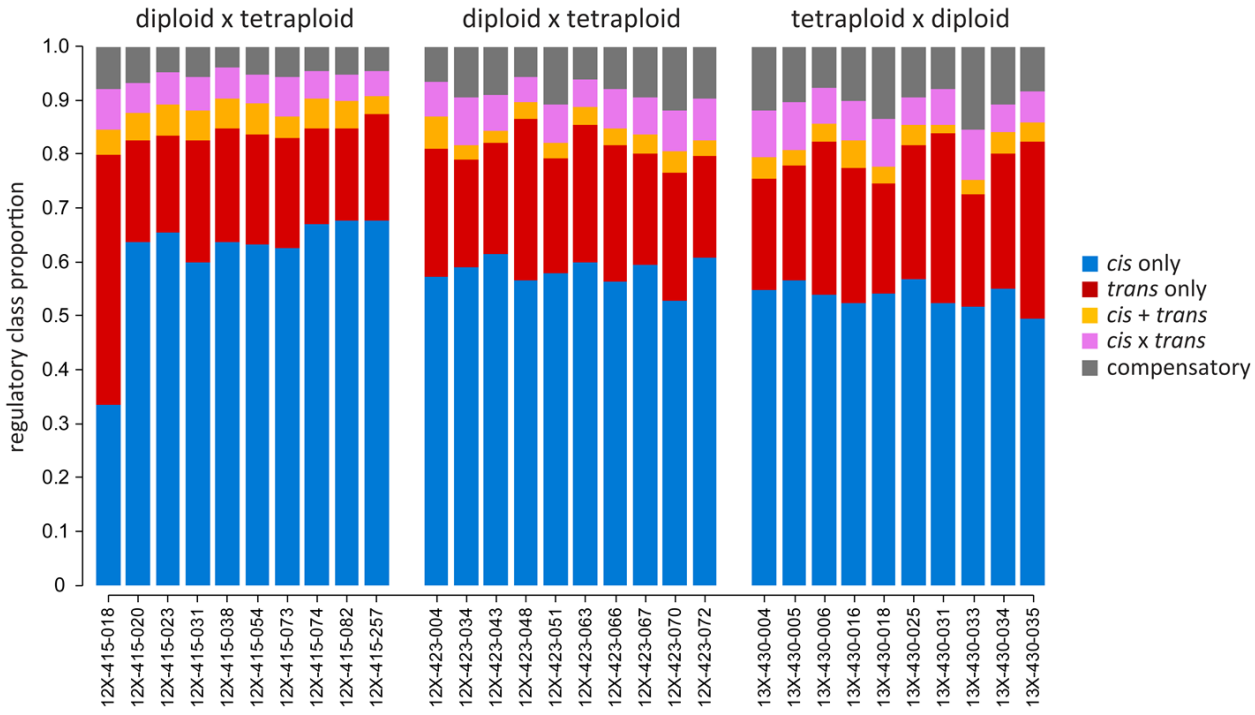

Fig. 7. Patterns of regulatory divergence of allele-specific expression in triploid progeny, with strong *cis* control followed by *trans* regulation.

Task 2.3. Across-species analysis of gene regulatory networks.

Analysis of gene regulatory networks is still underway, since the new assembly and annotation of high quality PacBio assemblies of *Salix purpurea* became available only recently. WGCNA will be used to study genes that are co-regulated across progeny individuals and may be controlled by factors that are correlated with heterosis.

Objective/Task 3. Validation of specific instances of non-additive expression and allelic imbalance in hybrids and triploids. The Sequenom MassArray instrument that was originally proposed for allele-specific expression assays has been removed from the Cornell Life Sciences Core Laboratory and is no longer available for our project. We will be using real-time quantitative PCR for allele-specific expression to confirm patterns of gene expression in field-collected tissue. Tissue for RNA extraction has been collected from the field and will be used to validate differential expression of genes showing interesting patterns of differential expression.

Products Delivered Throughout the Entire Project:

Publications:

Serapiglia, M.J., Gouker, F.E., and Smart, L.B. (2014) Early selection of novel triploid hybrids of shrub willow with improved biomass yield relative to diploids. ***BMC Plant Biol.*** 14:74, doi:10.1186/1471-2229-14-74.

Carlson, C.H. and Smart, L.B. (2016) Electrical capacitance as a predictor of root dry weight in shrub willow (*Salix*; Salicaceae) parents and progeny. ***Appl. Plant Sci.*** 4: DOI: 10.3732/apps.1600031

Carlson, C.H., Choi, Y., Chan, A., Serapiglia, M.J., Town, C.D., and Smart, L.B. (2017) Dominance and sexual dimorphism pervade the *Salix purpurea* L. transcriptome. ***Genome Biol. Evol.*** 9:2377-2394. DOI: 10.1093/gbe/evx174.

Presentations:

Smart, L.B. "Breeding shrub willow for improved yield and biofuels conversion efficiency", Cornell University, Dept. of Biological and Environmental Engineering, Ithaca, NY, Sept. 20, 2012. *Invited departmental seminar*.

Smart, L.B., Gouker, F.E., Serapiglia, M.J., Town, C.D., Tang, H., Buckler, E.S., Elshire, R.J., Mitchell, S.E., DiFazio, S., Rodgers-Melnick, E., Tuskan, G.A., Carlson, J.E., Miller, R.O., Volk, T.A., and Fabio, E.S. "Development of genomic resources and novel species hybrids for the genetic improvement of shrub willow feedstock crops" Sun Grant Initiative National Conference, Oct. 3, 2012, New Orleans, LA. *Poster presentation*.

Smart, L.B. "Breeding shrub willow as a sustainable feedstock crop for biofuel production", *International Symposium on Forest Environments and Low Carbon Green Growth*, Thirtieth Anniversary Symposium of the College of Forest and Environmental Sciences, Kangwon National University, Chuncheon, South Korea, Oct. 16, 2012. *Invited plenary speaker*.

Smart, L.B. "Breeding shrub willow for improved yield and biofuels conversion efficiency", Korea Forest Research Institute, Suwon, South Korea, Oct. 18, 2012. *Invited department seminar*.

Smart, L.B., Gouker, F.E., Serapiglia, M.J., Town, C.D., Tang, H., Buckler, E.S., Elshire, R.J., Mitchell, S.E., DiFazio, S.F., Rodgers-Melnick, E., Barry, K., Tuskan, G.A., Carlson, J.E. "Genomic and mapping resources for the genetic improvement of shrub willow feedstock crops", 9th Biennial Short Rotation Woody Crops Operations Working Group Conference, Oak Ridge, TN, November 5-8, 2012. *Oral presentation*.

Smart, L.B. "Breeding shrub willow for improved yield and biofuels conversion efficiency", Annual Meeting of NEERA1005 Multistate Research, Education, and Extension Project, Geneva, NY. Dec. 19, 2012. *Oral presentation.*

Gouker, F., Serapiglia, M., Tang, H., Town, C., Buckler, E., Mitchell, S., Elshire, R., Hyma, K., Rodgers-Melnick, E., DiFazio, S., Barry, K., Lindquist, E., Schmutz, J., Tuskan, G., Smart, L. "Sequencing and Assembly of the *Salix purpurea* Genome and Transcriptome to Improve Shrub Willow for Biomass Production", International Plant and Animal Genome Conference (PAG-XXI), Jan. 13, 2013, San Diego, CA. *Oral presentation*.

Smart, L.B. "Genomic approaches to improve yield and biofuels conversion efficiency of shrub willow", Cornell University, Dept. of Plant Biology, Ithaca, NY, Feb. 1, 2013. *Invited departmental seminar*.

Smart, L.B., Gouker, F.E., Serapiglia, M.J., Town, C.D., Tang, H., Buckler, E.S., Elshire, R.J., Mitchell, S.E., DiFazio, S., Rodgers-Melnick, E., Tuskan, G.A., Carlson, J.E., Miller, R.O., Volk, T.A., and Fabio, E.S. "Development of genomic resources and novel species hybrids for the genetic improvement of shrub willow feedstock crops", 2013 Genomic Science Annual Contractor-Grantee Meeting/USDA-DOE Plant Feedstock Genomics for Bioenergy Program Meeting, Feb. 24-27, 2013, Bethesda, MD. *Poster and short oral presentation*.

Smart, L.B. "Breeding to improve yield and sustainability of shrub willow bioenergy crops", Ninth International Conference on Biomass for Energy, Ukraine Bioenergy Association, Kiev, Ukraine, Sept. 25, 2013. *Oral presentation*.

Craig H. Carlson, Fred E. Gouker, Michelle J. Serapiglia, Haibao Tang, Vivek Krishnakumar, Christopher D. Town, Gerald A. Tuskan, Daniel Rokhsar, David M. Goodstein, Shengqiang Shu, Kerrie W. Barry, Erika A. Lindquist, Ran Zhou, Stephen DiFazio, and Lawrence B. Smart. "Annotation of the *Salix purpurea* L. Genome and Gene Families Important for Biomass Production", USDA-DOE Feedstock Genomics Investigator's Meeting and International Plant and Animal Genome Conference (PAG-XXII), Jan. 12-15, 2014, San Diego, CA. *Poster presentation*.

Carlson CH, Tang H, Krishnakumar V, Tuskan GA, Rokhsar DS, Goodstein DM, Shu S, Barry KW, Lindquist EA, DiFazio SP, Smart LB. "Assembly and functional annotation of the *Salix purpurea* L. chloroplast genome", Plant and Animal Genome Conference XXII, San Diego, CA; January 12-14, 2014. *Poster Presentation*.

Smart, L.B., Gouker, F.E., Serapiglia, M.J., Fabio, E.S., Carlson, C.H. "Breeding triploid hybrids of shrub willow with improved yield and biomass composition", Short-Rotation Woody Crops Operation Working Group Biannual Meeting, July 17-19, 2014, Seattle WA. *Oral presentation*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Michelle J. Serapiglia, Christopher D. Town, Haibao Tang, Vivek Krishnakumar, Stephen P. DiFazio, Eli Rodgers-Melnick, Ran Zhou, Shengqiang Shu, David M. Goodstein, Kerrie W. Barry, Erika A. Lindquist, Jeremy Schmutz, and Gerald A. Tuskan "Genomic approaches to improve yield and biofuels conversion efficiency of shrub willow", International Poplar Symposium VI, July 20-24, 2014, Vancouver, BC, *Invited oral presentation*.

Smart, L.B. "Breeding of triploid hybrids of shrub willow", Presentation to plant breeders and willow crop management team, Lantmännen Lantbruk and SalixEnergi Europa, Oct. 29, 2014, Svalöv, Sweden. *Invited oral presentation*.

Smart, L.B. "Genomics assisted breeding of triploid hybrids of shrub willow", Willow Research Symposium, Oct. 30, 2014, Swedish Agricultural University (SLU), Alnarp, Sweden, *Invited oral presentation*.

Carlson CH, Chan AP, Town CD, Serapiglia MJ, Smart LB. "Transcriptome analysis of diploid and triploid species crosses of shrub willow (*Salix* spp.). The United States Department of Agriculture - Department of Energy Feedstock Genomics Annual PI/PD Meeting, San Diego, CA; January 12, 2015. *Poster Presentation*.

Carlson CH, Chan AP, Choi Y, Serapiglia MJ, Tang H, Krishnakumar V, Town CD, Smart LB. "Transcriptome variation in shrub willow (*Salix*) hybrids", Forest Tree Workshop, International Plant and Animal Genome Conference XXIII, San Diego, CA; January 13, 2015. *Invited Symposium Talk*.

Smart, L.B. "Genomics assisted breeding of triploid hybrids of shrub willow for bioenergy", Horticulture Section, School of Integrative Plant Science, Cornell University, March 16, 2015, Geneva, NY. *Departmental seminar*.

Carlson CH, Gouker FE, Zhou R, DiFazio SP, Smart LB. "High-resolution mapping of biomass-related traits in an intraspecific F_2 shrub willow (*Salix purpurea* L.) family", Forest Tree Workshop, International Plant and Animal Genome Conference XXIII, San Diego, CA; January 10, 2016. *Invited short talk*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Eric S. Fabio, Chase R. Crowell, Christine D. Smart, Ran Zhou, Felipe R. Montes, John E. Carlson, Armen R. Kemanian, and Stephen DiFazio "Breeding and sustainability of shrub willow for marginal lands in the Northeast US"

International Plant and Animal Genome Conference (PAG-XXIV), Jan. 9, 2016, San Diego, CA. *Invited oral presentation*.

Carlson CH, Gouker FE, Zhou R, DiFazio SP, Smart LB. "High-resolution mapping of biomass-related traits in an intraspecific F_2 shrub willow (Salix purpurea L.) family", School of Integrative Plant Science and the Field of Food Science and Technology Symposium, Ithaca, NY; February 20, 2016. Poster Presentation.

Carlson CH, Gouker FE, Zhou R, DiFazio SP, Smart LB. "Mapping biomass traits in shrub willow (*Salix purpurea* L.)", School of Integrative Plant Science and the Field of Food Science and Technology Symposium, Ithaca, NY; February 20, 2016. *Invited Symposium Talk*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Chase Crowell, Chris D. Smart, Ran Zhou, Stephen DiFazio, Agnes Chan, Chris D. Town, and Gerald A. Tuskan "Molecular breeding of shrub willow for production of bioenergy", MERC Project Annual Meeting, Madaba, Jordan, April 1, 2016. *Invited oral presentation*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Eric S. Fabio, Chase R. Crowell, Christine D. Smart, Ran Zhou, Felipe R. Montes, John E. Carlson, Armen R. Kemanian, and Stephen DiFazio "Breeding and sustainability of shrub willow for marginal lands in the Northeast US" 8th Annual New York State Biotechnology Symposium, Syracuse, NY. May 19, 2016. *Invited oral presentation*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Eric S. Fabio, Michelle J. Serapiglia, Agnes Chan, Yongwook Choi, Chris D. Town, Eli Rodgers-Melnick, Luke Evans, Steve DiFazio. "Breeding for heterosis in shrub willow bioenergy crops" Dept. of Plant Biology, Rutgers University, East Rutherford, NJ. Nov. 4, 2016. *Invited departmental seminar*.

Smart, L.B., Fred E. Gouker, Craig H. Carlson, Eric S. Fabio, Michelle J. Serapiglia, Agnes Chan, Yongwook Choi, Chris D. Town, Eli Rodgers-Melnick, Luke Evans, Steve DiFazio. "Breeding for heterosis in shrub willow bioenergy crops" Plant Pathology and Plant-Microbe Biology Section, SIPS, Cornell University, Geneva, NY. Nov. 15, 2016. *Invited departmental seminar*.
Carlson CH, Choi Y, Chan AP, Serapiglia MJ, Town CD, Smart LB. "Differential expression, regulatory divergence, and sex dimorphism pervade the shrub willow (*Salix* spp.) transcriptome", The United States Department of Agriculture - Department of Energy Feedstock Genomics Annual PI/PD Meeting, Washington, DC; January 7, 2017. *Poster Presentation*.

Carlson CH, Choi Y, Chan AP, Serapiglia MJ, Gouker FE, DiFazio SP, Town CD, Smart LB. "Differential expression, regulatory divergence, and sex dimorphism pervade the shrub willow (*Salix* spp.) transcriptome", Forest Tree Workshop, International Plant and Animal Genome Conference XXV, San Diego, CA; January 15, 2017. *Invited Symposium Talk*.

Carlson CH, Choi Y, Chan AP, Town CD, Smart LB. "Geneva, NY - Where the willows are always above average", New York State Agricultural Experiment Station Research Symposium, Cornell University, Geneva, NY; June 23, 2017. *Invited Symposium Talk*.

Smart, L.B., **Eric S. Fabio, Fred E. Gouker, Craig H. Carlson.** "Breeding for heterosis in triploid hybrid shrub willow bioenergy crops", Nanjing Forestry University, Nanjing, China, July 31, 2017, *Invited symposium seminar*.