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USDA and DOE

Project Director Meeting

AFRI Plant Genome, Genetics and Breeding Program

AFRI Plant Breeding and Education Program

USDA-DOE Feedstock Genomics for Bioenergy Program

Town and Country Resort and Convention Center

San Diego, CA

January 13, 2011

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USDA - NIFA and DOE - BER
Project Director Meeting
Friday January 13, 2012
San Diego, California
Town and Country Resort and Convention Center
Meeting House - Sunset Room / Sunrise Room

7:30 a.m.	**Arrival, registration, set-up posters and light refreshments**
8:00 a.m.	**Session I: AFRI Plant Genome, Genetics and Breeding**
8:00 – 8:10 a.m.	Ed Kaleikau – Introduction
8:10 – 8:40 a.m.	Dave Neale – Genomics-Based Breeding in Forest Trees: Are We There Yet and Why We Need a Reference Genome to Finish the Job
8:40 – 9:10 a.m.	Eduard Akunov – Genome-wide Patterns of SNP Variation in Wheat: Tools and Resources for Breeding and Studying Genetics of Agronomic Traits
9:10 – 9:40 a.m.	Eric Jackson – Oat SNP Development and Identification of Loci Affecting Key Traits in North American Oat Germplasm Using Association Genetics
9:40 a.m.	Refreshments
10:00 a.m.	**Session II: AFRI Plant Breeding Education**
10:00 – 10:10 a.m.	Liang-Shiou Lin – Introduction
10:10 – 10:40 a.m.	Matias Kirst – Advanced Pine Breeding through Association Genetics and Biotechnology
10:40 – 11:10 a.m.	Seth Murray – Improving Maize Against Aflatoxin and Drought: Translational Plant Breeding, Education, and Extension
11:10 – 11:40 a.m.	Allen Van Deynze – An Integrated Approach to Breeding Resistance to Phytophthora Capsici in Pepper
11:45 a.m.	**Lunch (on your own) and view posters**
1:00 p.m.	**Session III: USDA-DOE Feedstock Genomics for Bioenergy**
1:00 – 1:10 p.m.	Cathy Ronning – Introduction
1:10 – 1:40 p.m.	Gautam Sarath – Building Improved Crown and Rhizome Transcriptomes to Evaluate Seasonal Changes in Switchgrass Populations with Divergent Winter Survival
1:40 – 2:10 p.m.	Andrew Paterson – Accelerating the Domestication of Miscanthus for Biofuel Production
2:10 – 2:40 p.m.	Pamela Green – Genome-Wide Analysis of miRNA Targets in Brachypodium and Biomass Energy Crops
2:45 p.m.	Refreshments
3:00 – 5:00 p.m.	**Poster Session**
3:00 – 4:00 p.m.	Posters I (Even #'s)
4:00 – 5:00 p.m.	Posters II (Odd #'s)
5:00 p.m.	Take down posters and adjourn

Speaker Abstracts

(In Presentation Order)

Genomics-Based Breeding in Forest Trees: Are We There Yet and Why We Need a Reference Genome to Finish the Job

David B. Neale

Dept. Plant Sciences, University of California, Davis

Efforts to develop genetic marker based approaches to breeding forest trees began in the late 1980s. Approaches based on first generation of markers, allozymes, were not feasible due to the very limited number of markers (<50). The first DNA-based markers, RFLPs, brought more hope as moderately dense genetic maps could be constructed to scan the genome and map quantitative trait loci (QTLs), however, this approach could not be brought to application in tree breeding due to low levels of linkage disequilibrium (LD) in forest tree breeding populations and recombination with each generation. The next generation of DNA markers based of the polymerase chain reaction, RAPD, AFLP and SSR, did not solve the LD and recombination problem, even though more markers were available and throughput increased.

The situation began to change in the early 2000s with the availability of automated DNA sequencing technology and single nucleotide polymorphisms (SNPs). Now association studies could be performed where SNPs within candidate genes controlling complex traits could be identified and thus "solving" or minimizing the LD and recombination limitation. This approach has been used to find candidate gene SNPs associated to a broad array of quantitative traits of interest (wood properties, growth, abiotic stresses and disease resistance). However, like QTL mapping before, individual SNP x trait associations only account for a small proportion of the variation (generally less than 2-3% of the total phenotypic variance) and the total variation explained by all markers is generally less than 50%. In human genetics, this situation is called the "missing heritability" and there has been great debate over whether this problem can ever be solved in humans. In a few agricultural systems however, notably dairy cattle, researchers are now accounting for nearly all the heritable variation.

Efforts to realize genomics-based breeding in conifer tree improvement in the US have culminated under The Conifer Translational Genomics Network Coordinated Agricultural Project (CTGN CAP). Marker-trait associations were validated in applied breeding programs and education and training programs were delivered to tree breeders. The CTGN CAP was the first to apply the Illumina Infininium SNP-genotyping technology that is now being used in the breeding of most major crops such as corn, wheat, tomato, potato, and many more. The only remaining limitation of the genomics-based approach is that the total variation controlling traits can still not be accounted for due to the inability to scan the entire genome. The solution to this problem is a reference genome sequence for which funding was awarded by NIFA in 2011. *De novo* reference genome sequencing is underway for loblolly pine, sugar

pine, and Douglas-fir. The full genome sequence will soon allow tree breeders to practice genomics-based breeding using genetic variation from the entire genome (genomic selection), and thus minimizing or even eliminating the missing heritability problem in forest trees.

These projects were supported by Agriculture and Food Research Initiative Competitive Grants no. 2009-85606-05680 and 2011-67009-30030 from the USDA National Institute of Food and Agriculture.

Genome-Wide Patterns of SNP Variation in Wheat: Tools and Resources for Breeding and Studying Genetics of Agronomic Traits

Eduard Akhunov

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Genome-wide analysis of genetic variation is a powerful tool for detecting marker-trait associations in diversity panels and mapping populations. Genome-scale genotyping data for such projects can be generated either by using high-throughput assays detecting allelic variation in a predefined set of SNP loci or by direct sequencing. Large-scale discovery of gene-associated SNPs has been performed in polyploid wheat by deep sequencing of 27 wheat cultivars' transcriptomes. Seven million 454 sequence reads and 11,000 full-length cDNAs were used to build Reference Transcripts (RT) for mapping RNA-seq data. SNP calling performed by aligning >750 million Illumina reads to RT resulted in discovery of 182,000 variable sites with 85-90% validation rate. Three custom high-throughput SNP genotyping assays (1,536-, 9,000- and 50,000-plex) based on Illumina BeadArray and Infinium platforms have been developed.

The 9,000 iSelect assay was used to genotype ~12,000 wheat lines in total generating 60 million data points for breeding lines, cultivars, landraces, wild relatives and progenies of mapping populations. Out of ~9,000 SNP assays 95% produced high-quality genotype calls with up to 70% being polymorphic in a worldwide collection of wheat cultivars with a minor allele frequency >0.05. A high-density genetic map of >5,000 SNPs was constructed and is currently being used for developing a wheat reference map integrating Genotyping-by-Sequencing (GBS) tags, SSRs and DArT markers. SNP genotyping data was used to investigate the patterns of linkage disequilibrium and genetic variation in a worldwide sample of wheat lines. The regions of wheat genome potentially involved in local adaptation were identified using

population genetics approaches. Genotyping of additional 10,000 wheat accessions with 50,000 SNP iSelect assay in collaboration with international community is planned for spring 2012.

The utility of developed SNP assays for genome-wide association mapping (AM) was tested by investigating marker-trait associations between genotypic data generated for a winter wheat population and more than 20 agronomic traits. Our study showed that the density of genotyping achieved for this population provided high power (>85%) to detect associations for traits with moderate levels of heritability (≥0.5).

An alternative approach to SNP detection relies on direct sequencing of complexity reduced genomic libraries prepared either by restriction digestion or by selective capture of genomic regions of interest. We demonstrated that sequence capture approach is an efficient tool for studying sequence variation in polyplod wheat genome. A SureSelect assay to capture 3.5 Mb of target sequence based on 3,500 cDNAs was designed and used to re-sequence exonic regions in diploid, tetraploid and hexaploid wheat. Approaches for variant discovery in polyploid genome by sequence capture were developed. RT sequences generated in the project were contributed into the design of 100 Mb Nimblegene exon capture assay that has been developed in collaboration with international community and currently is being tested.

Our project developed community genomic resources and tools for accelerated analysis of marker-trait associations critical for advancing wheat genetics and breeding. A collaborative network involving international research groups, organizations and industry was established to perform analyses of genetic variation in wheat populations at global scale.

***Project website**:* http://wheatgenomics.plantpath.ksu.edu/snp/

Oat SNP Development and Identification of Loci Affecting Key Traits in North American Oat Germplasm Using Association Genetics

Eric Jackson

USDA ARS Aberdeen, ID

Cultivated oat (*Avena sativa*) has many desirable traits including grain characteristics which, when consumed, reduce the primary risk factors leading to Coronary Heart Disease. Despite this well substantiated claim, development of oat varieties with superior agronomic, milling, and nutritive traits has been hindered. The primary reason for this is the complexity of the oat genome coupled with the lack of human and monetary resources applied to research and development. Since the early 1980's, oat research groups in North America have fallen from 29 to 11. Likewise, US oat acres have fallen from 14 million in 1988 to just over 2 million in 2008. Due to these alarming trends and the proven health

benefits of consuming oat-based products, the USDA NIFA AFRI sponsored research objectives formulated by a global group of oat scientists and US stakeholders to: i) develop SNP and GBS resources for oat ii) genotype relevant breeding material and populations, and identify QTL affecting key traits improving food quality and resistance to biotic stresses, iii) provide breeder centric data management and visualization tools to the oat research community through the "The Avena Toolbox", and iv) develop predictive assays for agronomic, milling, and nutritive traits. To date, the AFRI sponsored project has developed two innovative SNP selection approaches to efficiently develop robust assays from *in silico* results. This work has yielded 2,183 transcriptome-, DArTome-, and AACC genome specific-SNP assays which have been used to construct the first consensus map of hexaploid oat. The map has been physically anchored to chromosomes using a novel chromosome deficient hybrid approach resulting in a completely anchored consensus map. Capitalizing on this tremendous accomplishment, both bi-parental and association mapping studies have revealed genomic regions and specific markers linked to key agronomic qualities and disease resistance. Utilizing the transcriptome-based SNP loci bookending each genomic region as anchors, we have identified candidate genes conferring resistance to crown rust (b*Zip, Leucoanthocyanidin reductase*) and barley yellow dwarf virus (*adenosylhomocysteinase*) in these studies using a comparative genomics approach. Sequence information from oat and rice is currently being used to "fish out" the complete genomic sequence of both candidate crown rust genes which will be used to validate their effect on the crown rust phenotype and develop predictive assays.

Beyond the current research accomplishments of this project, we have effectivly reversed the declining trend of oat research critical mass. Currently, over 30 research laboratories across the globe housing five post doctoral fellows and four master's students are engaged in different research objectives of the project. This new trend is a critical step in accomplishing our long term goal of increased production and consumption of higher quality oats with heighten nutritive value.

Advanced Pine Breeding Through Association Genetics and Biotechnology

Matias Kirst

Project Director: Matias Kirst[1,2]

Co-PDs: John M. Davis[1,2], Dudley Huber[1], Timothy Martin[1], Gary F. Peter[1,2]

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The US southern states generate almost one fifth of the global wood supply, mostly from loblolly pine (*Pinus taeda*). Wood productivity in pine plantations is limited by water and nitrogen availability, and diseases such as fusiform rust and pitch canker are a common threat. However, improving these characteristic through traditional breeding is logistically complex, expensive, and time-consuming – *a single pine breeding cycle takes one or more decades*. Thus, new breeding strategies that incorporate advanced genomic approaches and biotechnology need to be conceived to support forestry in the U.S. Critical is also the training of plant geneticists with the capability to integrate classic and modern genetic improvement approaches to this effort.

The research objectives of this project have two components. In the first we are performing deep allele sampling of over 10,000 genes in association populations of loblolly pine, to identify and verify causative alleles of large effect on biomass growth, wood quality, disease resistance and drought tolerance. To characterize these genes, we are using sequence capture and re-sequencing for SNP detection in 900 genotypes. A set of probes was initially designed to capture 6.6Mbp of the 21.7Gbp loblolly pine genome, and capture efficiency was shown to exceed 70%. Currently, capture of target genes has been largely completed and sequencing is under way for future association genetic analysis. We also expanded the use of the sequence capture approach to: (1) characterize the genetic diversity of the 10,000 genes in a set of 24 unrelated individuals of loblolly and slash pine that represent the diversity of the two species; (2) map the majority of these genes by analyzing a segregating population, and (3) detect gene copy number variants to identify associations with phenotypes. A second objective of this project is the application of genomic selection to accelerate breeding of loblolly pine. In the first application of this approach in a conifer species, we demonstrated that selection efficiency gains of over 100% can be achieved for growth and disease resistance traits. Prediction models are also being used to identify the most suitable crosses (i.e. mate allocation) for broad adaptability, and resistance to pathogens.

As part of the education objectives of this project we are training graduate students in a customized curriculum with courses in genetics and breeding, molecular biology and field-based physiological genetics. In that effort we have recruited four graduate students who are developing skills in the full scope of tools available to breeders, including tree population management and improvement, genetic transformation, phenotypic analysis of transgenic trees and association populations, statistical analysis and information management.

Improving Maize Against Aflatoxin and Drought: Translational Plant Breeding, Education, and Extension

Seth C. Murray

Seth C. Murray [1], Mike Kolomeits[2], Thomas Isakeit[2], Gerald De La Fuente[1], Ivan Barerro[1]

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Texas is the 12th largest producer of maize (*Zea mays* L.) in the US and the largest outside of the Midwest. However, maize yield in Texas has not kept pace with the national average. Texas maize production is most limited by abiotic stress from drought and biotic stress from the fungus *Aspergillus flavus* which produces the potent carcinogen, aflatoxin, in the grain. Both stresses are predicted to increase throughout the US and world as a result of global climate change and will challenge feeding the growing population. Importantly, responses to both stresses are complex traits and no major genes for resistance are known for either. Recent basic research demonstrated that mutants of two maize lipoxygenase (LOX) gene family members can mediate quantitative variation for drought tolerance (*LOX4*) and aflatoxin resistance (*LOX5*) in maize. We hypothesized that natural variants of these genes exist in maize that might be useful for breeding. Leveraging a widely used association mapping panel we have sequenced within these two genes (which share 95% homology) and found ~10 novel alleles but relatively low genetic diversity. These association lines are being directly tested in the field as hybrids using two versions of tester line Tx714: one with the mutant *lox4* allele and one with the mutant *lox5* allele which allows direct testing of the panel's native alleles. Using an experimental design exposing these hybrids to *A. flavus* and drought, wide variations in aflatoxin resistance and drought tolerance were observed in phenotypic data collected on 470 hybrids this year. The collected data from this year and 2012 will be used in association mapping study to formally test *LOX4* and *LOX5* alleles. Additional traits such as epicuticular wax and seedling drought tolerance, as well as techniques such as fourier-transformed near-infrared reflectance spectroscopy (FT-NIRS) are also used towards these challenges.

In addition to researching technological solutions to these problems it is critical to educate the next generation of students and inform producers of the problems caused by aflatoxin and drought. Specifically we are highlighting the immense technical challenges, and the increasing array of tools to solve these problems. Towards graduate education we developed a distance education class in molecular quantitative genetics in plant breeding, conducted a study abroad trip for students to attend CIMMYT, provided three undergraduate research internships, and supported the training of two graduate research assistants. In 2011, to assist growers, we conducted experiments on four farms to evaluate new technologies for managing aflatoxin under drought conditions. Around aflatoxin we also developed two extension publications, and had direct contact with over 1000 growers and others involved in agriculture at 13 county meetings and field days. As an integrative approach, we have

developed a website to serve as a portal for knowledge on the related issues of drought and aflatoxin, connecting stakeholders to opportunities in plant breeding, pathology, and sound scientific solutions for their problems.

An Integrated Approach to Breeding Resistance to Phytophthora Capsici In Pepper

Allen Van Deynze

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The US pepper industry in 2011 was valued at $772.5 M. One third of the land planted to peppers is to chili (hot-type) peppers. One of the biggest problems for the pepper industry is *Phytophthora capsici* (Pc) in which all commercial varieties suffer yield losses despite good management practices and available landraces with high levels of resistance. Moreover, breeding resistance to Pc is complicated by the dynamic array of races of *Phytophthora* found in fields over time and variable resistance across plant varieties and tissue types. A high density map with 3892 markers was generated in a set of recombinant inbred lines derived from the highly resistant *Capsicum annuum* accession Criollo de Morelos-334 (CM334) and Early Jalapeño. These lines are being systematically screened for root rot resistance and leaf blight against a set of isolates defined by differential analyses. We will report on the integration of ultra high density mapping to introgress QTL for resistance to *Phytophthora capsici.* Concurrently, our research has been integrated into undergraduate and K-12 programs where we have reached over 1500 students to-date through experiential learning.

Building Improved Crown and Rhizome Transcriptomes to Evaluate Seasonal Changes in Switchgrass Populations with Divergent Winter Survival

Gautam Sarath

Gautam Sarath, Nathan A. Palmer, Aaron J. Saathoff, Christian M. Tobias, Paul Twigg, Kenneth P. Vogel and Madhavan Soundararajan.

The crown and rhizome transcriptome of an upland tetraploid switchgrass cultivar cv Summer well adapted to the upper-Midwest was investigated using the Roche 454-FLX pyrosequencing platform. In all approximately 1 million reads consisting of 216 million bases were assembled into 27,687 contigs and 43,094 singletons. Analyses of these sequences revealed minor contamination with non-plant sequences (< 0.5 %), indicating that a majority were for transcripts coded by the switchgrass genome. Blast2Go comparisons resulted in the annotation of ~65 % of the contig sequences and ~40 % of the singleton sequences. Contig sequences were mostly homologous to other plant sequences, dominated by matches to the *Sorghum bicolor* genome. Singleton sequences, while displaying significant matches to *Sorghum bicolor*, also contained sequences matching non-plant species. Comparisons of the 454 dataset to existing EST collections resulted in the identification of 30,177 new sequences. These new sequences coded for a number of different proteins and a selective analysis of two categories, namely peroxidases and transcription factors resulted in the identification of specific peroxidases and a number of low-abundance transcription factors expected to be involved in chromatin remodeling. KEGG maps for glycolysis and sugar metabolism showed high-levels of transcripts coding for enzymes involved in primary metabolism. The assembly provided significant insights into the status of these tissues, and broadly indicated that there was active metabolism taking place in the crown and rhizomes at the post-anthesis, seed maturation stage of plant development.

We have used this 454 assembly with datasets obtained from the Illumina platform to build a more robust hybrid transcriptome. This hybrid assembly was used to perform RNA seq analysis of mRNA obtained from two contrasting switchgrass populations, namely cv Summer, an upland tetraploid cultivar with good winter hardiness, and cv Kanlow, a lowland tetraploid cultivar with limited adaptation to the central great plains. Three biological replicates of crown and rhizome tissues obtained from field-grown plants post seed set were used to discover statistically valid differences in transcript abundances between Summer and Kanlow plants at this specific harvest date. Gene set enrichment analysis (GSEA) generated based on KEGG pathway assignments in Blast2GO, yielding a total of 142 gene sets. GSEA revealed that 9 gene sets were over-represented in Summer while 41 gene sets were over-represented in Kanlow (at an FDR level of 0.20). Enzyme activities and metabolite levels will be quantitated for select pathways to validate transcriptional profiling.

Accelerating the Domestication of Miscanthus for Biofuel Production

Andrew Paterson

Already among the most productive temperate grasses for lignocellulose production, scientific breeding of *Miscanthus* is only beginning and substantial improvements are probable. However, there exists

inadequate information about aspects of *Miscanthus* genome biology that have both practical and fundamental importance. *Miscanthus* has a basal set of 19 chromosomes, versus the 10 that is characteristic of much of the Saccharinae. However, the true relationship of the 19 *Miscanthus* chromosomes to those of other Saccharinae, important to translating genomic data from models such as sorghum, was not previously known. We have produced cDNA-derived SSR-based genetic maps of *Miscanthus sacchariflorus* Robustus and *M. sinensis*, the progenitors of the promising cellulosic biofuel feedstock *M*. × *giganteus.* The maps reveal a whole genome duplication affecting the *Miscanthus* lineage after the divergence of subtribes Sorghinae and Saccharinae. While the present maps provide for many early research needs, additional markers are also needed to improve map density and to further characterize the structural changes of the *Miscanthus* genome since its divergence from sorghum and *Saccharum*. Still unknown are important parameters such as levels and patterns of homoeologous gene duplication; and the types and frequencies of genetic polymorphism in both diploids and polyploids that are central to *Miscanthus* improvement. We are exploring pilot data from massively-parallel sequencing studies to craft appropriate experiments to substantively address these questions.

Many early priorities for *Miscanthus* improvement are 'domestication traits' about which there exists much information in sorghum and/or sugarcane, and for which the locations of controlling genes/QTLs often correspond across divergent grasses. To facilitate identification of 'hotspots' for Saccharinae QTLs for specific traits, we have developed a CMAP resource that contains QTL data from 26 populations of sorghum or sugarcane for 71 different traits, and is aligned to the sorghum reference maps and genome sequence. The resource will go public in the near future. Further, starting from gene/QTL locations in sorghum and other cereals, we are empirically testing corresponding *Miscanthus* loci to try to accelerate discovery of DNA markers diagnostic of biomass yield determinants including flowering time, stalk dimensions, axillary bud production/rhizome expression, and flooding tolerance. Intensive phenotyping of two populations is in progress: (a) *Miscanthus lutarioriparia x M. sinensis*: *M. lutarioriparia*, a Chinese endemic riparian grass, grows to 6-7 meters in height, with long rhizomes and flood tolerance necessary to its riparian habit. *M. sinensis* is a bunch-type grass not tolerant of submergence. An F_2 population of about 200 individuals is being phenotyped and genetically mapped. (b) *C*rosses between early, northern *M. sacchariflorus* robust (from the Amur river region) and *M. sinensis* led to the 'Amuri' populations, of which some individuals have been selected as early cold-tolerant hybrid cultivars for northern temperate regions. An F_1 population (parents are highly heterozygous) of about 300 individuals is being phenotyped, with a subset of these being the basis of the genetic maps described above. A scaffold of DNA markers are being placed on the remainder of the population for QTL mapping. Early associations of traits with phenotypes have been found.

Genome-Wide Analysis of miRNA Targets in Brachypodium and Biomass Energy Crops

Pamela J. Green

University of Delaware

miRNAs are small, endogenous RNAs that post-transcriptionally regulate gene expression in nearly all eukaryotic systems. In plants, miRNAs can serve as major regulators of development, stress responses, metabolism, and other processes through the miRNA-guided cleavage of specific target RNAs. While miRNAs and their target interactions are well-characterized in systems such as *Arabidopsis* and rice, less is known about the targets of miRNAs in others including temperate grasses and potential bioenergy crops. In this study, we use next-generation sequencing technology to identify miRNAs in different tissues and following abiotic stress treatments of *Brachypodium distachyon,* a rapidly developing model system for temperate grasses and bioenergy crops. To identify the targets of these miRNA on a global scale, we use an approach called PARE (Parallel Analysis of RNA Ends) that facilitates the sequencing of 3' products of miRNA-guided target RNA cleavage. A total of more than 64 million reads were obtained from 12 small RNA libraries, resulting in an average of more than 1.4 million distinct genome-matched small RNA sequences per library. The analysis of this data identified more than 100 new, non-conserved annotated, and conserved miRNAs. These miRNAs were used to create a pipeline for miRNA target discovery from PARE data. Four PARE libraries have been analyzed with the pipeline, and PARE sequences were found for hundreds of target cleavage sites of the miRNAs identified under this project. More than 20% of these target cleavage sites matched the new Brachypodium miRNAs that were discovered. In addition, tissue-preferential cleavages by distinct miRNA family members have been identified using the PARE data. Our PARE data were also useful for identifying cleavages and small RNAs that initiate some of the phased small RNAs from Brachypodium that we previously reported (International Brachypodium Initiative, Nature 463:763-768,2010). This indicates that PARE is a powerful tool to identify miRNAs that trigger phased siRNA production, in addition to its major application to miRNA target identification and cleavage validation. Because miRNAs and their targets can form missing links in many important gene regulatory networks, the identification of miRNA-target RNA pairs in *Brachypodium* will help to better understand how small RNAs contribute to the regulation of genes and genomes. Future experiments to expand this analysis in Brachypodium and bioenergy crops will be discussed. This research was supported by the Office of Science (BER), U.S. Department of Energy.

Plant Genome, Genetics and Breeding Program Project Reports

(Alphabetical Order of Lead Project Director)

Single Nucleotide Polymorphism (SNP) Markers for High-Throughput Genotyping to Advance Genomic, Genetic and Breeding Research in Wheat

Eduard Akhunov

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Project website: http://wheatgenomics.plantpath.ksu.edu/snp/

Objectives and Accomplishments: The objectives of this proposal are: **1)** perform large scale SNP discovery in the transcriptomes of 8 US cultivars (year 1); **2)** use 4,608 SNPs for designing Illumina genotyping assays (year 1); **3)** use SNP genotyping assays for genotyping 96 US cultivars and 3 wheat CAP mapping populations for assessing the patterns of genetic diversity (years 2 and 3); **4)** develop publicly accessible SNP resource (years 2 and 3). The goals of all project objectives were successfully met.

We generated >**750 million** RNA-seq reads for transcriptomes of **13 US, 1 Chinese** and **13 Australian** wheat cultivars. Wheat Reference Transcriptome including **140,000** unigenes has been assembled for SNP discovery. Analysis of project data discovered more than **182,000** gene-associated SNPs that were deposited into our publicly accessible project SNP database. **A sequence capture assay** for targeted SNP discovery in wheat exons was designed (published in Genome Biology). Three high-throughput Illumina SNP genotyping assays including **1536, 9,000** and **50,000 SNPs** have been developed. Our project coordinated an international consortium that genotyped more than **12,000 wheat accessions** including 5,500 US wheat cultivars and breeding lines using 9000 SNP iSelect assay. We also collaborated with the

TCAP project on genotyping of additional 4,400 wheat lines. Five mapping populations were genotyped in our project and a high-density SNP genetic map including **>5000 SNPs** is developed. This SNP map is currently being used for developing a wheat reference map integrating Genotyping-by-Sequencing (GBS) tag, SSR and DArT markers. The 9000-SNP genotyping data was used to investigate the patterns of linkage disequilibrium in the wheat genome and perform genome-wide association mapping. The regions of the wheat genome involved in adaptation to local climatic conditions were indentified using population genetics approaches. Genotyping of an additional **10,000 wheat accessions** with **50,000 SNP assay** in collaboration with the international community is planned for spring 2012. High-density SNP map of wheat is being developed by mapping **182,000** wheat SNPs to chromosome-specific survey sequencing data in collaboration with the International Wheat Genome Sequencing Consortium (IWGSC).

Broad Impacts: The PDs of this project lead an International Wheat SNP Working Group (IWSWG) whose major goal is to facilitate the development of advanced open-access marker technologies based on SNPs. Our group developed three public genotyping assays (**1536, 9,000** and **50,000 SNPs**) and organized an international collaborative effort to genotype in total nearly 16,000 tetraploid and hexaploid wheat accessions including the mapping populations, landraces, breeding lines, cultivars and wild relatives. Several association mapping panels and mapping populations are currently being used for mapping genetic determinants of disease resistance, tolerance to biotic and abiotic stress factors. The **9000 iSelect assay** developed by our group has been used as a major genotyping tool in multiple national and international public and private projects involving Triticeae CAP, Canadian Triticum Advancement through Genomics, Monsanto, Pioneer, Syngenta and KWS.

Our **3.5 Mb exon capture assay** for targeted analysis of genetic variation in genic regions served as a prototype for developing a larger **100 Mb sequence capture** assay in collaboration with Nimblegen company. The transcriptome sequence data generated in our project provided >30% of 100 Mb Nimblegen capture assay which is currently being tested by our group.

Our reference transcriptome data is being used for annotating and predicting genes in the wheat genome in collaboration with the IWGSC. We are also collaborating with the IWGSC on the development of a high-density genetic variation map of wheat.

Deliverables:

Publications:

1. Saintenac C, Jiang D, **Akhunov E.** Targeted analysis of nucleotide and copy number variation by exon capture in allotetraploid wheat genome. Genome Biol 2011. 12:R88.
2. **Chao S**, Dubcovsky J, Dvorak J, Luo MC, Baenziger SP, Matnyazov R, Clark DR, Talbert LE, Anderson JA, Dreisigacker S, Glover K, Chen J, Campbell K, Bruckner PL, Rudd JC, Haley S, Carver BF, Perry S**, Sorrells ME, Akhunov E.** Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (Triticum aestivum L.). BMC Genomics 2010 11:727.

3. **E. Akhunov**, **S. Chao**, V. Catana, **D. See**, **G. Brown-Guedira**, **M. Sorrells**, A. Akhunova, **J. Dubcovsky**, C. Cavanagh and M. Hayden. New tools for wheat genetics and breeding: genome-wide analysis of SNP variation. Proceedings of BGRI Technical Workshop, June 13-16, 2011, St. Paul, Minnesota, U.S.A.
4. Kiani S, Akhunova A, **Akhunov E.** Application of next-generation sequencing technologies for genetic diversity analysis in cereals. Cereal Genomics II. Editors: Gupta PK and Varshney RK, Springer (*in press*).
5. **Akhunov E**, Sehgal S, Liang H, Wang S, Akhunova A, Kaur G, Li W, Forrest K, **See D**, Simkova H, Hayden M, Luo M, Farris J, Dolezel J, Gill B. Comparative analysis of orthologous genes in grass genomes reveals accelerated rates of alternative splicing and coding sequence evolution in polyploid wheat. Plant Cell (*submitted*).

Presentations in meetings and scientific symposia:

15 presentations were made at scientific meetings, workshops and symposia

Community Resources Generated:

1536-, **9000-** and **50,000**-plex Illumina SNP genotyping assays are developed and publicly accessible. **14,000** tetraploid and hexaploid wheat accessions including the mapping populations, landraces, breeding lines, cultivars and wild relatives have been genotyped with **9000 iSelect assay**. The genotyping data for 2,400 accessions have been deposited into the TCAP database. The project SNP database contains SNP data at >182,000 loci among 27 US and Australian cultivars http://wheatgenomics.plantpath.ksu.edu/snp/. The searchable wheat reference transcriptome database contains more than 488,000 assembled transcripts. The processed part of the next-generation sequence data produced for wheat transcriptomes is publicly available through NCBI Sequence Read Archive (SRA012746; http://www.ncbi.nlm.nih.gov/sra).

Other products/ outcomes:

The international wheat SNP working group website was created to provide updates on international SNP development projects and facilitate development of a community SNP resource (http://wheatgenomics.plantpath.ksu.edu/IWSWG/).

Training: S. Wang and V. Catana (postdoctoral res.): development of SNP discovery bioinformatical pipeline and processing of next-generation sequence data; S. Kiani (postdoctoral res.): high-throughput SNP genotyping data analysis and genome-wide association mapping experiments; M.M. Waghmare, S.G. Gudala, D. Jiang (computer science grad. students): development of project website and SNP database; Tristan Coram (postdoctoral res.): expression analysis and preparation of cDNA libraries.

Collaborations: The project established close connections with international wheat research groups (TCAP - US, BreedWheat - France, Monogram - UK, Australia, Canada, IWGSC), organizations (Bill and Melinda Gates Foundation, CIMMYT) and industry (Pioneer Hi-Bred, Syngenta, KWS, Biogemma).

Genome-Wide Selection to Introgress Exotic Dwarf-Corn Germplasm into U.S. Corn Belt Germplasm

Rex Bernardo

Project Director: Rex Bernardo, University of Minnesota, bernardo@umn.edu
Project Website: http://agronomy.cfans.umn.edu/exotic_corn_genomewide_selection.html

Objectives and Accomplishments: Corn (*Zea mays* L.) hybrids have become higher yielding, shorter, and better adapted to high plant population densities. A dwarf plant stature may lead to higher corn productivity at very high plant population densities, expanded areas for production, and benefits in crop rotation, weed management, and control of soil erosion. In this research, cheap and abundant single nucleotide polymorphism (SNP) markers are used to introgress useful traits from dwarf corn into adapted corn inbreds. Specific objectives are to (1) develop corn germplasm that combines the high grain yield of conventional (i.e., non-dwarf) corn and the reduced stature and adaptability to high plant population densities of dwarf corn; and (2) determine if, as indicated by theoretical studies, genomewide selection is useful for the rapid improvement of an adapted × exotic cross for multiple traits.

In 2011, we continued cycles 1 to 4 of genomewide selection in a greenhouse, as well as phenotypic backcrossing of the dwarfing trait into two non-dwarf inbreds. Based on the SNP marker results, we are predicting continued progress from genomewide selection for grain yield and plant height in the two populations undergoing selection. But continued responses to selection are not predicted for grain moisture (now 18% in one population and 23% in the second population), stalk lodging (<3% in both populations), and root lodging (<2% in both populations). Field trials will be conducted in 2012 to evaluate the actual progress from selection.

Broad Impacts: We highlight two broad outcomes. First, greenhouse observations indicated that flowering dates and maturities have changed with phenotypic backcrossing but not with genomewide selection. This result suggests that genomewide selection is useful for avoiding unwanted side effects associated with backcrossing major genes. Second, we confirmed that prediction accuracy in genomewide selection is affected by marker density, training population size, and trait heritability. However, traits differed in their prediction accuracy even when heritability, training population size, and marker density were all kept constant. This result suggests that empirical evidence and experience on the predictability of a trait are needed in designing suitable training populations in genomewide selection.

Deliverables:
Publications:

Combs, E.E., and R. Bernardo. 2012. Joint effects of population size, marker density, heritability, and trait architecture on accuracy of genomewide predictions in biparental populations. Theor. Appl. Genet. (submitted)

Oral/Poster presentations:

Combs, E.E., and R. Bernardo. 2011. Genomewide selection to introgress exotic dwarf-corn germplasm into U.S. corn belt germplasm. 53rd Annu. Maize Genet. Conf., 17-20 Mar. 2011, Saint Charles, IL.

Combs, E.E., and R. Bernardo. 2011. Genomewide selection to introgress exotic dwarf-corn germplasm into U.S. corn belt germplasm. Monsanto Poster Session, 3 Nov. 2011, Saint Paul, MN.

Community resources generated:

The cycle 0 genotypic and phenotypic data and cycles 1 to 3 genotypic data are now available for the dwarf × non-dwarf crosses. These data will be deposited in *MaizeGDB* when data from all cycles of marker-based selection also become available in 2012. The cycle 0 data, however, are available from the PD upon request.

Training:

Combs, Emily. Ph.D. Applied Plant Sciences (Plant Breeding/Molecular Genetics), Univ. of Minnesota, 2009-present. The research described herein comprises Ms. Combs's Ph.D. dissertation, and she contributed the journal article and oral presentations described above.

In addition to her Ph.D. studies and research, Ms. Combs went on a summer internship in 2011 at CIMMYT in Mexico to learn more about computational aspects of genomewide selection from the CIMMYT biometry team.

Collaborations:

Dr. Dahu Chen (DNA Landmarks, Quebec, Canada), SNP marker analysis

Dr. Xiuling Zhang (Pioneer Hi-Bred Intl., Eau Claire, WI), Ph.D. committee member

Dr. Jose L. Crossa (CIMMYT, Mexico), mentor during summer 2011 internship

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Understanding the Mechanisms that Define Cereals: Unraveling the Function of Lineage Specific Genes within the Poaceae

C. Robin Buell

Project Director: C Robin Buell, Michigan State University, buell@msu.edu

Co-PDs: Ning Jiang, Michigan State University, Ning Jiang, jiangn@msu.edu

Objectives and Accomplishments:

The objectives of this proposal are to determine the function of a conserved yet phylogenetically distinct set of genes in the cereals, the Conserved Poaceae Specific Genes (CPSGs). The CPSGs are broadly conserved within the Poaceae but are not present (as detected by sequence similarity) in 150 other species in the Plant Kingdom. Understanding the function of the CPSGs has the potential to provide insight into unique biological processes in the Poaceae and potentially, phenotypes important to agriculture.

The specific objectives of this project are:
Obj. 1: Re-compute the set of CPSGs using newly released plant genome sequence and annotation data.
This has been completed. Using our rice genomic annotation, along with sequence from 22 Poaceae species, we identified 2,087 rice genes that are specific to the Poaceae family, of which, 455 have an ortholog in maize, sorghum and Brachypodium. Of these, only 34 % of the rice loci have expression support and we have performed quantitative expression analysis using RNA-seq technology to further characterize expression patterns in these four Poaceae species.

Obj. 2: Use whole transcriptome sequencing approaches to generate expression profiles across a comparable set of developmental time points/stages for four Poaceae species with genome sequence (rice, sorghum, maize, and Brachypodium).
We have generated whole transcriptome sequence data for 48 tissue samples from these four core Poaceae species that represent a developmental time series through floral and seed development. These data have been analyzed. One manuscript is in press, one is in preparation.

Obj. 3: Perform phylogenetic and expression analyses within and between the four species to identify orthologous CPSGs that are expressed in comparable developmental stages across all four Poaceae species for targeted functional genomic studies.
We have identified sets of genes across the Poaceae that have conserved expression patterns in floral and seed tissues.

Obj. 4: Test the function of CPSGs using rice mutant, knockout, knockdown, and/or over-expression lines using a phenotyping panel with a standardized set of metrics for floral/seed development and yield.
We have initiated construction of over-expressing lines for a subset of CPSGs. We have cloned full-length cDNAs for 6 rice CPSGs into transformation vectors. We have generated 6 transgenic Arabidopsis lines over-expressing target CPSGs. We have generated 5 transgenic rice lines over-expressing target CPSGs. We have screened 55 tagged insertion rice lines and identified putative mutants in leaf, seed and flower development; molecular characterization of these insertion lines is in progress.

Obj. 5: Provide sequence, expression, orthology, and phenotype data to the community through project websites, archiving in GenBank, and deposition in established biological databases.

We have released all maize and rice RNA-seq data. The sorghum and Brachypodium RNA-seq data are scheduled to be released in December 2011.

Broad Impacts:

Our transcriptome dataset will provide insights into coordinated expression patterns during reproductive development in a core set of Poaceae species.

Deliverables:

Publications: One manuscript is in press; a second manuscript is in preparation.

Oral/ Poster Presentations: Plant and Animal Genome, 2011

Community Resources Generated: Transcriptome data made available to community.

Training:

Malali Gowda, Postdoctoral Fellow, was trained in genomics. Dr. Gowda was responsible for tissue and RNA isolation and cDNA library construction.

Rebecca Davidson, Postdoctoral Fellow, was trained in bioinformatics. Dr. Davidson performed bioinformatic analyses of the RNA-seq data.

Lina Quesada, Postdoctoral Fellow, was trained in bioinformatics. Dr. Quesada has been involved in the phenotyping, cloning, and bioinfomatic analyses.

Brieanne Vaillancourt, Research Technologist, was trained in cDNA library construction, next generation sequencing, cloning, and large-scale data management.

Haining Lin, Postdoctoral Fellow, was trained in next generation sequencing analysis, specifically in using transcript reads to measure gene expression.

Daniel Aaron, Undergraduate Professorial Aide, was trained in solution/media preparation, plant care, phenotyping, and plant transformation methods. Mr. Aaron has become familiarized with floral and seed development in Arabidopsis to phenotype transgenic lines expressing rice CPSGs.

Byron Oja, Undergraduate Laboratory Aide was trained in solution/media preparation, plant care, and phenotyping methods.

Terin Budine, Undergraduate Research Intern was trained in plant care, molecular techniques, and phenotyping methods.

Melanie Hardin, Undergraduate Laboratory Aide was trained in plant care, molecular techniques, and phenotyping methods.

Advancing the Barley Genome

Tim Close

Project Director: Timothy J. Close, University of California, Riverside, CA, timothy.close@ucr.edu

Co-PDs: Stefano Lonardi, University of California, Riverside, CA, stelo@cs.ucr.edu; Gary J. Muehlbauer, University of Minnesota, St.Paul, MN, Gary.J.Muehlbauer-1@tc.umn.edu; Jeffrey L. Bennetzen, University of Georgia, Athens, GA, maize@uga.edu)

Project websites: www.harvest.ucr.edu, www.harvest-web.org, www.harvest-blast.org

Objectives and Accomplishments: Due to improvements in sequencing costs and very efficient new algorithm designs for sequence deconvolution, the objective of assigning genes to BACs was increased from 2000 BACs to the entire available set of 14,763 minimal tiling path BACs. This includes an estimated 75% of all expressed genes. The strategy to sequence nearly all genes in Morex barley was modified to include whole-genome shotgun sequencing in addition to two previously planned gene enrichment methods. Several data releases have been made. Enhancement of the physical map now includes an upgrade of the genetic linkage map. Laboratory and computational method development for "combinatorial sequencing" of BACs based on a shifted transversal design, to assign specific genes to BACs, has come to fruition. More details are below.

Objective 1: Determine the sequences of nearly all Morex barley genes, including 5' and 3' flanking regions using high Cot, methyl filtration and cDNA sequencing. This will provide reference sequences for gene-BAC deconvolution, and for annotation in general.

Shotgun, whole-genome sequencing. Sequencing of a high Cot fraction was replaced with shotgun whole-genome sequencing. A total of 31X depth of coverage sequence has been generated using Illumina GAII and HiSeq 2000, including standard paired-end sequences from 350-400 bp libraries and mate-pair sequences from 2 kb, 3 kb and 5 kb libraries. Assemblies were produced using SOAPdenovo, with postings periodically on our BLAST server at www.harvest-blast.org and a sequence retrieval tool at http://harvest-web.org/utilmenu.wc. Version 0.04 of "The Barley Genome" contained 1.13 Gb of assembled sequences with N50 = 1860 and BLASTN hits of e-20 for >90% of all previously identified barley gene sequences.

Methyl filtration. Sequencing of hypomethylated partially restricted DNA to enrich for expressed genes has been accomplished, with analysis in progress.

Transcriptome. Prior to this project only ~68,000 Morex EST sequences were publicly available, accounting for ~1/3 of genes identified by barley ESTs. This project generated ~4 Gb of cDNA sequences using Illumina GAII from 5 Morex cDNA libraries, each composed of mRNA from multiple tissues, developmental stages or conditions. Exact matches are needed to assign short-read Morex BAC sequences to reference sequences. Assemblies were produced using Velvet/Oases, posted periodically for BLAST on www.harvest-blast.org and retrieval from http://harvest-web.org/utilmenu.wc. Version 0.02 of "Barley Transcriptome" contained BLASTN hits of e-25 for 88% of previously known Morex ESTs, and about 27% previously unknown barley transcript sequences.

Objective 2: Sequence BACs from a minimal tiling path (MTP) of previously-identified gene-bearing BACs to assign many more genes to BACs. This will enhance the physical map.

Combinatorial Sequencing. The full set of gene-bearing MTP BACs is ~14,763 clones. Seven sets of combinatorial pools of BACs, 91 pools per set (637 pools), were prepared. Pools were made for 3-decodability using a shifted transversal design. All pools were examined GoldenGate assays to create a short list of genes present in each pool, then sequenced. The usual sequencing method involved 13 multiplexed pools per lane, for a total of 91 pools per 7-lane flow cell. Sequences were deconvoluted to specific BACs using a program that we named HASHFILTER (developed initially by simulation using rice genome sequences), then each set of reads for each specific BAC was assembled using Velvet. The average depth of coverage for each BAC was ~150X with a mean coverage of ~75%. Highly repetitive sequences are not resolved to individual BACs by HASHFILTER.

Enhancement of the Genetic Map. SNP data from 373 individuals in 4 doubled haploid (DH) mapping populations previously used for a 2943-SNP 975-bin genetic map were reexamined and combined with additional data to constitute a new dataset from 1133 individuals in 1 RIL and 9 DH populations. An improved genetic map containing 2994 SNPs in 1163 bins has been produced (Munoz et al. 2011) from this larger dataset, improving the anchoring of the physical to the genetic map.

Broad Impacts and Collaborations: Information from this project supports genetic mapping and genome databasing objectives of a new USDA-AFRI NIFA Coordinated Agricultural Project, "Improving Barley and Wheat Germplasm for Changing Environments" and those of the International Barley Genome Sequencing Consortium.

Deliverables:

Oral/ Poster Presentations: USDA-AFRI, BarleyCAP and Barley Workshops preceding and during the Plant and Animal Genome meeting, San Diego, California, Jan 2010 and 2011; Seminar "Advancing the Barley Genome" at U Minnesota, Nov 2, 2010.

Publication: Munoz et al. 2011. An improved consensus map ….. Plant Genome 4:238.

Community Resources Generated (consistent with Objective 3 - data release):

HarvEST:Barley contains a SNP-based genetic map and the identity of anchored BACs. Available through www.harvest-web.org or for Windows from http://harvest.ucr.edu.

The barley physical map available at http://phymap.ucdavis.edu/barley shows FPC assembly information.

GrainGenes http://wheat.pw.usda.gov shows the new genetic map.

Draft genome and Morex transcriptome assemblies are BLASTable at www.harvest-blast.org; sequences can be retrieved from http://harvest-web.org/utilmenu.wc.

Training: Matthew Alpert, UC Riverside (UCR) undergraduate in Computer Sciences, genome assembly; Denisa Duma UCR PhD student in Computer Sciences, gene-BAC deconvolution algorithms; Steve Wanamaker, UCR Programmer, transcriptome assembly and HarvEST:Barley upgrades; Yaqin Ma, PhD researcher and Raymond Fenton, Technician, library production for BAC and genomic DNA sequencing; Maria Munoz, U Minnesota, post-doc, enhanced barley genetic map.

The Douglas-Fir Climate Change Transcriptome Observatory (CCTO) for the Pacific Northwest

Richard Cronn

Project Director: Richard Cronn, USDA Forest Service Pacific Northwest Research Station, Corvallis, OR, rcronn@fs.fed.us

Co-PDs: Brad StClair, USDA Forest Service Pacific Northwest Research Station, Corvallis, OR

Peter Dolan, University of Minnesota-Morris, Morris, MN

Dee Denver, Department of Zoology and Genetics, Oregon State University, Corvallis, OR

Project website: http://www.fs.fed.us/pnw/olympia/silv/ccto/index.html

Objectives and Accomplishments:

We merged reciprocal transplant studies of Douglas-fir that simulate extreme climate change with transcriptome sequencing to identify genes/pathways that are climate-responsive. Our objectives include: (1) defining a Douglas-fir needle transcriptome; (2) measuring daily and seasonal transcriptome variation in cold-, mesic- and warm adapted trees; (3) evaluating seasonal phenotypic variation in garden trees; and (4) relating transcriptome variation to weather, growth and phenology. The identification of climate responsive elements will improve our odds at identifying genes that contribute to adaptation in this genomically complex (36 Gbp/2C) tree, and enhance our ability to manage forest resources in the face of climatic uncertainty.

Goal 1: Our Douglas-fir transcriptome v.1 reference was assembled using 2,950,471 454/Roche reads. This strand-oriented draft included 25,002 isogroups, 38,589 contigs and 102,623 singletons totaling 53.6 Mbp. A v.2 reference has been constructed using 500 Gbp of Illumina RNA-seq data from CCTO trees, and will be released in January, 2011. ***100% complete***

Goal 2: To define seasonal variation, we collected tissues at 26 time points over one growing season (Oct. 2010 – Oct. 2011). RNAs have been isolated from all collection dates (888 total), and RNA-seq libraries are being constructed (228 planned samples). Diurnal transcriptome variation is being evaluated from an experiment that used a 4 hr sampling frequency and 48 hr duration (48 samples). To date, 96 RNA-seq libraries have been sequenced. ***60% complete***

Goal 3: Temperature, humidity, precipitation, soil moisture (5 cm - 50 cm) and irradiance were measured during the study. Bud phenology (spring burst; fall set) and radial growth were measured at 2d – 14d intervals. Leaf reflectance was measured at one time. ***100% complete***

Goal 4: Efforts to relate transcriptome variation to diurnal patterns, weather and phenotypic data are underway using our v.2 transcriptome reference. Initial efforts focus on diurnal variation and spring bud burst (7 weeks). Analysis of gene co-expression and interaction will be initiated when the complete annual transcriptome data set is available. ***10% complete***

Broad Impacts: The CCTO contributes to basic science, as RNA-seq and phenotypic data form the basis of a long-term 'transcriptome observatory' that will be resampled to evaluate the link between genotype, weather, and growth. Our transcriptome reference will play a central role in functional gene characterization for the USDA-funded Douglas-fir Genome Reference. Our study strengthens ties to applied forestry, as private industry has contributed land and resources to this study.

Deliverables:

Publications:

R. Cronn, B. Knaus, A. Liston, J. Maughan, M. Parks, J. Syring, J. Udall. 2012. Simplifying the complex: targeted sequencing approaches for plant population and evolutionary studies. American Journal of Botany (in press).

Oral/ Poster Presentations:

B. Knaus, P. Dolan, D. Denver, R. Cronn. 2012. Transcriptome dynamics in the dormancy-spring growth transition of Douglas-fir needles. Plant and Animal Genomes conference, San Diego, CA [Oral - invited].

B. Knaus, R. Cronn, et al. 2012. The Douglas-fir Climate Change Tanscriptome Observatory. Plant and Animal Genomes conference, San Diego, CA. [P]

R. Cronn, B. Knaus, et al. 2012. Western Forest Transcriptome Survey: Applying genomic discoveries towards understanding genome responses to climate change. Plant and Animal Genomes conference, San Diego, CA [P]

J. Yu, B. Knaus, et al. 2011. Another step for conifer genomics: a Douglas-fir transcriptome. Plant and Animal Genomes conference, San Diego, CA [Oral - invited]

T. Jennings, B. Knaus, et al. 2011. High-throughput conifer microsatellite recovery using multiplexed massively parallel sequencing. Plant and Animal Genomes conference, San Diego, CA. [P]

R. Cronn, B. Richardson, J. Wright. 2011. Western Forest Transcriptome Survey: Applying genomic discoveries towards understanding genome responses to climate change in western forests. Gordon Conference, Bar Harbor, ME [P]

R. Cronn. 2010. Next Generation sequencing: Applications. Botanical Society of America, Providence, RI [Oral – invited]

Community Resources Generated:

Our strand-oriented, annotated v.1 Douglas-fir Transcriptome Reference is available on our web site, and the v.2 reference will be available in 1/2012. Assembled references and 36 (raw) RNA-Seq files have been shared with the Pine Genome Reference Sequence project for public distribution (http://pinegenome.org/pinerefseq/). Laboratory methods for RNA-seq and informatic software developed by our group are available through the OpenWetWare project (http://openwetware.org/wiki/Cronn_Lab).

Training: (a) Undergraduate/post-baccalaureate training in RNA isolation, RNA-seq construction, field measurements**:** *Katherine Alderman, Teague Green, Tara Jennings, Kelly McDonald, Laura Mealy (Oregon State U)**;** Jennifer Swanson (Colorado State U).* (b) Graduate student training in RNA-seq construction and data analysis: *Prabin Bajgain (Brigham Young U); Matthew Parks (Oregon State U); Amy Ross-Davis (U Idaho).* (c) Post-doctorate and visiting scientist training in RNA-seq construction and data analysis: *Brian Knaus, Eriko Muira (US Forest Service); Katy Hayden (UC-Berkeley); Laura Beaver, Melanie Marine (Oregon State U); Hardeep Rai (Utah State U); Kristine Pilgrim, Bryce Richardson, Jessica Wright (US Forest Service); Karen Mock (Utah State U.).* We provided training materials and presentations at the "Botany 2010: Next-Generation Sequencing Workshop" in Providence, RI. The symposium attracted 55 participants, and it will be repeated in 2012.

Collaborations: We maintain active collaborations with several forest genetics and genomics groups, including: (a) Dr. Glenn Howe (Conifer Translational Genomics Network, USDA-CAP) and Dr. Jeff Dean (Conifer Community Sequencing Proposal, JGI) to develop the CCTO Transcriptome reference and SNP resources for Douglas-fir; (b) Dr. David Neale (Conifer Translational Genomics Network and Pine Reference Sequence, USDA-CAP) to develop the Douglas-fir genome reference; and (c) Drs. Bryce Richardson and Jessica Wright (Western Forest Transcriptome Survey, USDA Forest Service) to study the link between seasonal weather variation and climate on gene expression in a diversity of woody plants.

Translating Solanaceae Sequence Diversity and Trait Variation into Applied Outcomes through Integrative Research, Education, and Extension

David Douches

Project Director: David Douches (douchesd@msu.edu), Michigan State University

Co-PDs: Robin Buell (buell@msu.edu), Michigan State University; Walter De Jong (wsd2@cornell.edu), Cornell University; David Francis (francis.77@osu.edu), Ohio State University; Allen Van Deynze (avandeynze@ucdavis.edu), University of California, Davis; Lukas Mueller (lam87@cornell.edu), Cornell University; Alexandra Stone (stonea@hort.oregonstate.edu), Oregon State University

Project Website: http://solcap.msu.edu

Objectives and Accomplishments:

The SolCAP project was initiated September 2008 under grant no. 2008-55300-04757 of the USDA/NRI and is currently funded through USDA/NIFA/AFRI grant no. 2009-85606-05673.

Objective 1. Create an education program to train graduate students in genome-based breeding.

Graduate curriculum has been developed and delivered to students. Curriculum includes a graduate course in bioinformatics for plant breeding students http://www.oardc.ohio-state.edu/tomato/HCS806/HCS806.htm; data analysis with R (http://www.oardc.ohio-state.edu/tomato/hcs825/HCS806_R/HCS806_R_main.htm USERNAME: oardcwin\hcs825 PASSWORD: Genetics825); a graduate course "breeding with molecular markers" that was jointly delivered between Cornell and Ohio State University; and "Introduction to Scripting and Statistics for Genetics Data Management" (PLBR 4092, Cornell). The information delivered in these courses has been integrated into the plant breeding curriculum at The Ohio State University under "Advanced Plant Breeding" (HCS 7825), and at Cornell under PLBR 4092. SolCAP educational materials are also being recognized as a tool to train international audiences.

In 2011, workshops focused on working with phenotypic data, R programing and BLUPs, integrating molecular data generated with the Illumina chips and an introduction to SGN tools. Furthermore, workshops were broadcast as webinars to make them accessible to participants who were not able to travel to the meetings. These webinar sessions were recorded and are currently accessible at http://solcap.msu.edu/meetingsworkshops.shtml. Presentation slides and supporting documents (scripts for statistical analysis) are also available at this site and at http://pbgworks.org/tomato-workshop.

A "how to" methods in plant breeding series was initiated (http://www.extension.org/pages/60426). Three one-hour webinars delivered by members of SolCAP were broadcast live to an online audience. In addition, the broadcasts were recorded and uploaded to YouTube.

Objective 2. Amplify outreach efforts by developing an eXtension Plant Breeding Community of Practice (CoP) to develop continuing education material aimed at practicing plant breeders, their staff and seed industry professionals.

PB&GCoP was accepted by eXtension as a new Community of Practice March 3, 2010. The CoP has a space on the People website of eXtension (login required to view) http://www.extension.org/people/communities/363. PBGworks.org is the collaborative workspace that is being used for the CoP. Content is authored and reviewed there and then transmitted to

eXtension.org for publication. There are 5 CAP groups participating in the workspace, as well as a variety of lab groups and PBG eXtension content development and administrative groups. All SolCAP eXtension content is authored in the workspace. The PBGWorkspace currently has 209 people registered as members who represent 30 universities and federal agencies, 11 educational institutions outside of the USA, and 5 industry groups. The PBG CoP publicly launched 124 pages of content on January 15, 2011 (www.eXtension.org/plant_breeding_genomics). The PBG CoP now has 165 pages of public content. According to Google Analytics, the PBG eXtension website (www.eXtension.org/plant_breeding_genomics) has now had a total of 21,997 visits and 84,527 page views since launch on January 15, 2011. Views of PBG eXtension content in the last quarter account for 2.96% of eXtension activity for the 46 publicly launched communities.

SolCAP collaborated with the Conifer Translational Genomics Network CAP to coordinate peer-review and publication of a multi-part series of learning modules that is focused on the fundamentals of quantitative genetics in the context of molecular tree improvement. These learning modules aim to provide a comprehensive introduction to the fundamental background science and technology required to understand and incorporate genetic markers in applied tree breeding and resource management. Thirteen of 15 learning modules were peer-reviewed, voice-over narration of the modules was recorded, and the modules were published to YouTube (http://www.youtube.com/user/plantbreedgenomics#grid/user/7803AFC312DDB04A) and eXtension.org (http://www.extension.org/pages/60370/).

The PBG CoP launched a YouTube channel (http://www.youtube.com/user/plantbreedgenomics) to host webinar, workshop, and educational videos. The YouTube channel currently has 81 videos uploaded. The videos have been viewed 22,830 times. The channel currently has 66 subscribers.

Objective 3. **Collect standardized phenotypic data across multiple environments for tomato and potato.**

Field studies to collect standardized phenotypic data of tomato and potato lines from the germplasm panels were conducted in NC, NY, ID, MN, WA, WI, FL, OH and CA.
Some of the flexible funds were used to collect centralized sugar (sucrose, glucose and fructose), malic and citric acid and chip color data in a 4x potato mapping population and the potato diversity panel. Cooperator's guides for potato and tomato were developed and refined for the collection of the 2010/11 phenotypic data. 2011 potato field phenotype data is collected and post harvest phenotyping (sugar, chip color, malic acid, critic acid and vitamin C) is under way for the potato trials. A database of phenotypes for key traits across accessions for both commodities is now in place, accessible through SolCAP (http://solcap.msu.edu/) and Solanaceae Genome Network (SGN, http://solgenomics.net/).

The potato and tomato panel SNP genotyping is complete. We are initiating association mapping, germplasm diversity and population structure analyses, estimation of marker-based breeding values, and cross validation for genome-wide selection. The genotyping and phenotypes provide a guide for population structure and developing appropriate association

studies for subclasses. The potato and tomato communities have submitted mapping populations for SNP genotyping (three 4x and one 2x for potato and five for tomato). The community populations were approved by the SolCAP Executive Committee and these populations will enhance our mapping and validation efforts. Further validation will be done outside of SolCAP with funds by the public and private sector investment in the SolCAP-based SNP platforms for potato and tomato. As of fall 2011, tomato and potato SNP arrays have been purchased to genotype over 4,000 and 6,000 lines, respectively.

Objective 4. Develop extensive sequence data of expressed genes, and identify Single Nucleotide Polymorphisms (SNPs) markers distributed across the genome and associated with specific candidate genes for sugar, carbohydrate and vitamin biosynthetic pathways.
We have identified high quality SNPs for the development of the potato and tomato genotyping platforms. We have completed the design for the Infinium potato and tomato platforms. Both the potato and tomato SNP predictions have been validated using the Illumina BeadExpress platform. We developed a computational pipeline to identify SNPs using transcript sequences in conjunction with a reference genome and when coupled with two Sanger-derived Expressed Sequence Tag transcript datasets, we identified 62,576 non-redundant SNPs in tomato. Our computational pipeline was validated using the Illumina BeadXpress genotyping platforms with validation rates greater than 98.5%.

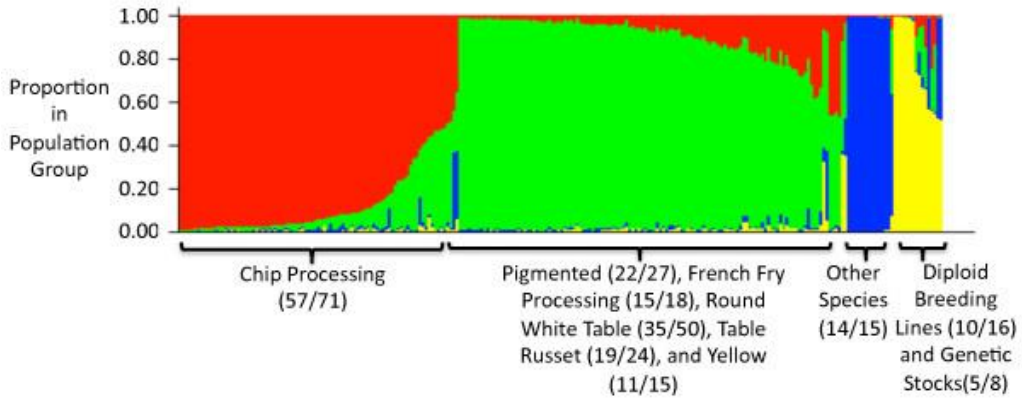
Over 69,000 high quality SNPs that meet the Illumina Infinium platform design criteria were identified for potato. Currently, ~8300 scoreable markers are represented on the potato Infinium array. Potato data has been shared with international collaborators (Scottish Crops Research Institute, SCRI) and the Potato Genome Sequencing Consortium to help anchor the potato genome sequence. Over 28,000 SNPs were identified in tomato, with 8,784 SNPs selected for development of an Illumina Infinium array. Of these, 7,720 SNPs passed production quality check and were subsequently scored on the tomato germplasm panel (n=489) representing cultivated varieties (processing, fresh market, vintage and landrace) and wild species. We obtained high quality genotype data (< 10% missing data) from 7,375 SNPs across the panel. Tomato data has been shared with collaborators, 3,700 SNPs have been placed on the *S. lycopersicum* x *S. pennellii* reference map, all markers have been placed on the physical map and have been integrated into SGN. Chromosome by chromosome patterns of linkage disequilibrium have been analyzed. The potato SNP manuscript has been published (BMC Genomics) and the tomato SNP manuscript will be submitted in December. The original proposal budgeted for 737,280 data points. The Illumina consortium provided the world-wide community with resources for a minimum of 22,800,000 data points.

For both SNP platforms, we identified candidate genes for sugar, carbohydrate, and vitamin biosynthetic pathways in potato and tomato as suggested by the research community and have solicited the community for genes to include in the potato and tomato genotyping platforms. In summary, there are 2769 SNPs in candidate genes, 508 SNPs in genetic markers and 6723 SNPs distributed throughout the genome based upon the reference genome scaffolds. We estimate that we are covering about 650Mb of the estimated 850Mb potato genome (the current reference genome is only about 727 Mb). For

tomato, 567 SNPs represent candidate genes, 1,470 SNPs were obtained from the community, and 270 scaffolds from the Heinz1706 reference genome assembly are represented in the final design.

Objective 5. Establish centralized facilities for genotyping a core set of SNP markers in standard germplasm panels in tomato and potato.

Genotypes for over 1152 potato and 480 tomato breeding lines with the Potato and Tomato Infinium platforms, respectively, have been assayed by SolCAP. Germplasm panels for tomato and potato genotypes were assayed by SolCAP from the community mapping populations in 2011. Preliminary population structure in potato has been defined with 82 SNPs with results published in BMC Genomics. A more extensive analysis with the 8303 potato SNPs are being worked on at this time and four manuscripts for potato and tomato will submitted for publication by January 2012.



Graphical display of population substructure for 248 genotypes at a population size *K* = 4. Population substructure was determined using STRUCTURE with 82 high quality SNP markers. Each genotype is represented by a vertical line. Color segments within the vertical line indicate the proportion of membership in each of the four population substructure groups. Population substructure groups are color-coded as population one (red), population two (green), population three (blue), and population four (yellow). Numbers in parenthesis indicate the number of genotypes with majority membership (greater than 50%) in each population group and the total number of genotypes for each market class.

Potato: The SolCAP project generated a custom three-cluster and five-cluster SNP file for the 8,303 Infinium chip by manual determining cluster positions for each marker within the Illumina GenomeStudio software. This custom cluster file, a coded GenomeStudio project file with the 443 clones described above for users to view the clusters on the germplasm they were determined with, and comments relating to the quality of each SNP (good, questionable, bad, distorted segregation) were released on the SolCAP website (http://solcap.msu.edu/potato_infinium.shtml) in December 2010 for the three-cluster file. From this analysis it was determined that 7,412 SNPs were of high quality, and the remaining 891 SNPs were of lower quality, had distorted segregation based on the mapping populations, or were of unusable quality. Using the mapping populations and diverse clones described

above, an additional five-cluster file was manually generated that defines the theta boundaries for each marker class for each of the 8303 potato SNPs. Although, a diverse set of germplasm was used, not all possible marker classes were represented for each SNP, and thus theta boundaries could not be defined. The breakdown for number of marker classes that could be defined is: 2,645 SNPs with five clusters, 858 SNPs with four of the five clusters, 945 SNPs with three of the five clusters, 583 SNPs with two of the five clusters, and 3,272 SNPs either only had one cluster or clusters could not be defined due to loose clusters that could not be distinguished. A custom perl script was also generated to convert the theta positions as outputted by GenomeStudio into meaningful genotype scores using these theta boundaries. The custom five-cluster file will be made publically available when the potato diversity panel manuscript is published.

In our russet tetraploid mapping population, only about 1/3 of the SNPs were nulliplex x nulliplex (homozygous and not segregating). Most of the SNPs in the validation assay are showing expected segregation ratios. We can call five genotypes (AAAA, AAAa, AAaa, Aaaa, aaaa) in over 30% of SNPs; therefore more SNPs will be available for mapping and QTL analysis. Reading SNP dosage will increase our power of analysis in the tetraploid segregation data.

Tomato: The 7,720 SNPs from the Infinium array have been mapped relative to both a standard genetic map (3,700 markers mapped through collaboration with Trait Genetics) and relative to the physical map via BLAST against the draft genome of H1706. The polymorphism rates of these SNPs were 63.3% for processing varieties, 86.8% for fresh market varieties, 81.4% for vintage varieties, and 96.9% for wild species. Graphing minor allele frequency (MAF) relative to genetic and physical positions revealed differences in haplotype blocks between market classes of cultivated tomato. Although the number of polymorphic markers within market classes and even within the breeding programs represented in the SolCAP panel exceeded expectations, recombination will restrict the informative use of all polymorphic markers. Analysis of LD decay based on genetic and physical distance on a chromosome-by-chromosome basis reveals extensive haplotype blocks within and between market classes. The extent of linkage disequilibrium (LD) was examined across each chromosome with LD decay ranging between 1.3-12.2 cM within processing varieties, 3.4-12.2 cM within fresh market varieties, and 0.6-21.7 cM within vintage varieties. These results have been incorporated into the strategy for Objective 6 below.

Objective 6. Address regional, individual program and emerging needs within the Solanaceae community through a small grants program.

To address emerging needs within the Solanaceae community, a call for mapping populations to be SNP genotyped with the Infinium arrays was sent to the potato and tomato communities in September 2010 and March 2011, respectively.

The potato community has developed two tetraploid and three diploid mapping populations for SNP genotyping for concordance to the draft genome and to validate marker linkages to major loci influencing vitamin and sugar content and validate QTL. These bi-parental mapping populations should complement the diversity panel and russet mapping population that SolCAP has genotyped and

phenotyped and should provide an opportunity for QTL validation. SNP Genotyping is now completed for potato.

For tomato, 12 populations were submitted by the community; six were selected for SNP genotyping. In assessing these populations, the SolCAP Executive Committee (EC) evaluated experimental design, data distribution, and data variability. These populations represent six public breeding programs and will assay yield, antioxidants, carbohydrates, late and early blight, virus, bacterial and fungal resistance, and flowering and reproduction traits. One population is being genotyped with support from seed industry/genetic service providers, thus further leveraging SolCAP resources. Two populations (one potato and one tomato) will be genotyped by sequencing (GBS) with Illumina. The potato GBS procedure is being optimized this fall. We feel that it is important that the small grants program is designed to vet community proposals. Through the review process, we will be able to direct translational research towards promising approaches. These represent a mixture of biparental mapping, introgression, MAS, and association mapping approaches.

Based on polymorphism of parents on the 7720 tomato Infinium chip and on observed recombination between markers, two 384 SNP panels were selected for processing and fresh market populations. SNPs in each panel were chosen based on genetic and physical locations in the genome.

Objective 7. Create integrated, breeder-focused resources for genotypic and phenotypic analysis by leveraging existing databases and resources at SGN and MSU.

We established a project web site (www.solcap.msu) which is a centralized resource for SolCAP participants and the Solanaceae breeding community. Modules to query phenotypic and genotypic data are being developed for SGN. These are cross-linked to eXtension Plant Breeding and Genomics hosting instructional modules and webinars on how to generate, analyze and integrate genomic data in breeding programs.
An integrated, breeder-focused resource for genotypic and phenotypic analysis was initiated by leveraging existing databases and resources at SGN and MSU. A breeder's toolbox for SGN is being modified to serve the tomato and potato breeding community.

SolCAP phenotype data were integrated into the Sol Genomics Network (SGN, http://solgenomics.net/) database. Tomato phenotype data from experiments in 2009 and 2010 are from four different locations with three different tomato categories (fresh market, vintage and processing) for a total of 13 experiments. A limited potato set has also been loaded (two 2009 experiments). Data is displayed in the form of pages for plant accessions, showing information on the origin, alternative synonyms, and other important metadata. Each accession has a list of member plots from all related field experiments, and a summary of the scored phenotypes.

The tomato Infinium chip sequences were mapped on the latest release of the tomato genome. All primer pairs mapped uniquely to this genome release using bwa mapping tool. The resulting genome coordinates were added to the tomato reference sequence map on SGN. Bulk download of the sequence information will be made available as soon as some additional quality checking of the data will be completed. Tutorials for using these tools have been or are under development and are linked

through eXtension.org. Database development and utility are concerns. There is a group dedicated to the development of the breeder's toolbox and this group is interacting via conference calls.

Broad Impacts:

We have almost complete participation of the US public potato and tomato breeding communities for the germplasm panels.

We have collaborated with Martin Ganal, Trait Genetics, Gaterslesben, Germany and Mathilde Causse, INRA, Avignon, France to incorporate some of their SNPs based on complementary germplasm into the community Tomato Infinium chip. We will be co-publishing an integrated genetic and physical map of tomato based on the SolCAP Infinium chip.

Strong interest from the international community to use the SolCAP potato SNP array. Researchers at The International Potato Center, Netherlands and Scotland have purchased the potato SNP array for population genotyping.

Use of the Infinium SNP genotyping system is allowing the calling of SNP allele dosage in tetraploid genotypes. Scoring for dosage will increase our power to genetically analyze populations of cultivated tetraploid potato.

Deliverables:

Four SolCAP Newsletters (http://solcap.msu.edu/news.shtml)

Felcher, K., J. Coombs, C. Hansey, A.N. Massa, C. R. Buell, D. Douches, J. Hamilton, R. E. Veilleux. 201X. Integration of Two Diploid Potato Linkage Maps with the Potato Physical Map. (submitted to PLOS One)

Hamilton, J.P., Sim, S-C., Stoffel, K., Van Deynze, A., Buell, C.R., Francis, D.M. 201X. Single Nucleotide Polymorphism Discovery in Cultivated Tomato via Sequencing by Synthesis. (submitted to Plant Genome)

Hamilton, J.P., Hansey, C.N., Whitty, B.R., Stoffel, K., Massa, A.N., Van Deynze, A. De Jong, W.S., Douches, D.S., and Buell, C. R. 2011. Single Nucleotide Polymorphism discovery in elite North American potato germplasm. BMC Genomics. 12:302

Pei, C., Wang, H., Zhang, J., Francis, D. M., Yang, W. 2011. Fine mapping and analysis of a candidate gene in tomato accession PI128216 conferring hypersensitive resistance to bacterial spot race T3. Theor. Appl. Genet. [Epub ahead of print]

Wang, H., Hutton, S.F., Robbins, M.D. Sim, S. C., Scott, J. W., Yang, W., Jones, J. B., Francis., D. M. 2011. Molecular mapping of hypersensitive resistance from tomato 'Hawaii 7981' to *Xanthomonas perforans* race T3. Phytopathology. 101(10):1217-1223.

Baldo, A. M., Francis, D. M., Caramante, M., Robertson, L.D., Labate, J. A. 2011. AlleleCoder: a PERL script for coding co-dominant polymorphism data for PCA. Plant Genetic Resources pp 1-3. Published online. http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=8333992

Sim SC, Robbins MD, Van Deynze A, Michel AP, Francis DM. 2011. Population structure and genetic differentiation associated with breeding history and selection in tomato (*Solanum lycopersicum* L.). Heredity. 106: 927-935.

Robbins, M.D., Sim, S-C., Yang, W., Van Deynze, A., van der Knaap, E., Joobeur, T., and Francis, D.M. 2011. Mapping and linkage disequilibrium analysis with a genome-wide collection of SNPs that detect polymorphism in cultivated tomato. Journal of Experimental Botany 62(6):1831-1845 . Epub ahead of print http://jxb.oxfordjournals.org/content/early/2010/12/30/jxb.erq367

Rodríguez GR, Muñoz, S. Anderson, C., Sim, S-C., Michel, A. Causse, M., McSpadden Gardener, B., Francis DM, van der Knaap E. 2011. Distribution of SUN, OVATE, LC and FAS in the Tomato Germplasm and the Relationship to Fruit Shape Diversity. Plant Physiology. 156: 275-285.

Twenty-eight Oral/ Poster Presentations

Community Resources Generated:

SolCAP cDNA Libraries Sequenced and High Throughput SNP analysis SolCAP

Germplasm Panels Elite potato germplasm was contributed from 16 programs across the U.S. as well as six international programs. The panel consists of 325 potato lines currently used by the community. A core collection of tomato germplasm has been assembled which includes 288 inbred lines from fresh market and processing tomato breeding programs.

SNP Development for the Illumina Infinium Platform for potato and tomato The SolCAP team led a consortium with our fellow Solanaceae scientists to develop potato and tomato Infinium SNP arrays through Illumina for the interrogation of SNPs in the respective genomes. These arrays are being using worldwide by the potato and tomato breeding and genetics communities.

SolCAP workshops have been organized and conducted for the tomato and potato communities in 2009, 2010 and 2011to provide a foundation for these communities to adopt genomics based analysis. Live webinars were conducted during the workshops to expand community outreach.

PBGworks SolCAP has developed PBGworks as a collaborative workspace developed using open source software. http://pbgworks.hort.oregonstate.edu/ which is being used for developing content on the PBG eXtension.org CoP.

SolCAP has created a project website which is a centralized resource for SolCAP participants. Visit us at http://solcap.msu.edu. SGN tools are available to breeders through the dedicated breeder's toolbox (http://solgenomics.net/breeders/).

Training: Seventeen post doc, staff and students have been trained.

Collaborations: As noted above, there are 17 strong collaborative arrangements among the public potato and tomato breeding and genetics community.

Project Evaluation

At the January 2009, 2010 and 2011 Plant and Animal Genome (PAG) meetings we held our advisory committee meetings. Verbal feedback and written reports from the advisory group have been used by the executive committee to further refine project activities and deliverables. Additional surveys were developed for use at the SolCAP workshops held at the Potato Association of America 2009, 2010, and 2011 annual meetings, the 2009 Tomato Breeders Round Table (TBRT), and the 2010 and 2011 Tomato Disease Workshops. Evaluation surveys were used to gather feedback on the specific workshop sessions as well as the workshop as a whole, including the extent to which advertised content was covered, learning objectives were met, presentation of theory and application was balanced, and participants gained new knowledge, as well as whether the technical level of presentations was appropriate for the audience and whether participants would recommend the workshop to others. Participants in these workshops for potato and tomato professionals rated the workshop sessions as being relevant to their work and effectively presented.

During 2011, in addition to the ongoing evaluation of SolCAP –sponsored workshops, an additional questionnaire was developed to obtain feedback from participants in the new series of educational webinars provided via eXtension.org through collaboration among NIFA CAP grants led by SolCAP. Through November 2011, responses from three webinars indicated that these educational events were viewed by participants as highly effective and aimed at an appropriate technical level for the audience.

Comparative Protein Networks Controlling Disease Resistance in Rice and Wheat

Jorge Dubcovsky

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Objectives and Accomplishments:

Objective 1. Translation of rice-rice to wheat-wheat protein interactions: We identified and cloned the wheat orthologous copies of rice NH1 (NPR1-like), Xa21, XB12, RAR1/SGT1/HSP90 and of 13 direct known rice interactors. Wheat orthologous copies of the known rice interactors were also tested for their ability to interact with wheat and rice central-node proteins using a yeast-two-hybrid system (Y2H). A general conservation of the protein-protein interactions was observed across species despite some differential gene family expansions that occurred after the rice-wheat divergence.

Both Xa21 and NPR1 wheat proteins were used to screen both a *Puccinia striiformis* (PST)-infected wheat cDNA library and a rice cDNA pool using a Y2H system. . We identified novel interactors that were not previously observed in rice, including potential PST effectors, which we are currently validating.

To understand better the potential interactions between wheat and stripe rust effectors we completed the sequence of the PST genome and made its sequence publicly available (see publications).

Objective 2. In planta studies: The first constructs for Xa21 and NH1 and their interactors have been completed for the *in planta* interaction validations using rice protoplasts and bimolecular fluorescence complementation (BiFC). For WKS1 we determined its chloroplast localization using WKS1::GFP transgenic wheat plants and validated *in vitro* incorporation inside isolated chloroplast.

Objective 3: *WKS1 interactors in wheat*: To validate *in planta* the WKS1 interactors identified by Y2H we generated stable wheat transformants expressing WKS1 fused to a C-terminal TAP-tag. Accumulation of the tagged protein has been recently confirmed by Western-blotting. We are currently carrying out tandem-affinity purification followed by mass-spectroscopy to identify co-purified *in planta* interactors.

Objective 4: *Genetic validation of interactions*: Truncation mutants for both *RAR1* and *Xb12* (both A and B genomes) were identified in our TILLING population and were backcrossed to the non-mutagenized control to reduce mutation load. Mutation in the two genomes were intercrossed and then combined to a near-isogenic line containing the *WKS1* disease resistance gene to study their effect on resistance. We also found a truncation mutation in the A genome copy of the WKS1-interacting protein VAMP-associated protein (VAP). For the B-genome VAP copy we found 18 mutations that produced amino acid changes. Mutations with the highest probability of altered protein structure and function were backcrossed to the non-mutagenized parent.

For the stem rust resistance gene *Sr35* we identified a candidate CC-NBS-LRR resistance gene by positional cloning and we are using it to screen a *P. graminis* infected wheat Y2H library to identify potential effectors interacting with *Sr35*. This reporting period we also completed and published the study of the epistatic interactions between the stripe rust resistance genes *Yr48* and *QYr.ucw-3BS*.

Results were disseminated through two publications and six presentations in scientific meetings.

Broad Impacts:

The stripe rust disease of wheat caused by the highly specialized fungal pathogen *Puccinia striiformis* f. sp. *tritici* (PST) has been responsible for large yield losses for centuries. Current epidemics of new aggressive races of PST that appear after the year 2000 pose significant threats to food security worldwide. In spite of its economic importance, the PST genomic sequence was not available. The most significant output of this year is the completion and publication of a draft sequence of the PST genome. In the first three months since its publication the PLoS One paper was viewed by 1304 users and downloaded 258 times, documenting the impact of this study. The paper provides access to > 90% of the genes of this pathogen, its repetitive sequence, and to an extensive list of candidate effector genes. This public information has the potential to accelerate a new wave of studies to determine the mechanisms used by this pathogen to infect wheat, and hopefully to reduce current yield loses caused by this pathogen.

This year we published the mapping of two major QTLs for resistance to the new virulent races of stripe rust and identified closely linked molecular markers that can be used by breeders to pyramid and accelerate the transfer of these resistance genes using marker assisted selection. These results have been published in the scientific journal Theoretical and Applied Genetics.

This year, in collaboration with Dr. Akhunov in Kansas State University we completed the cloning of the stem rust resistance gene *Sr35.* We are now using the cloned resistance gene as an entry point to identify the pathogen effectors recognized by this CC-NBS-LRR resistance gene. *Sr35 is* still effective against the new race UG99 from Africa that is now threatening major wheat growing regions of the world.

During this period we completed the cloning of 18 wheat orthologues of rice proteins involved in protein-protein interactions in four nodes of the rice disease interactome. Analysis of the interactions among the wheat proteins revealed that there is a substantial amount of conservation among the protein-protein interactions in the wheat and rice diseases interactome.

During this period we made substantial progress in our understanding of the resistance mechanism of the partial resistance gene *Yr36.* We confirmed that this gene is located in the chloroplast by both *in vitro* and stable wheat transgenic experiments and that there it interacts with a thylakoid-associated

ascorbate peroxidase which is responsible for the regulation or reactive oxygen (ROS) in the cell. Based on these results we hypothesize that *Yr36* alters ROS threshold induction levels of programmed cell and that this contributes to the detection of the presence of the pathogen.

An additional important output of this year experiments is the generation of TILLING mutants for the A and B genome copies of the RAR1 gene and their combination in a RAR1-null mutant, which will be a useful tool to classify disease resistance gene into RAR1/SGT1/HSP90 dependent and independent classes, initiating a functional characterization of the different resistance genes.

Deliverables:

Peer-reviewed publications:

Lowe, I., D. L. Jankuloski, S. Chao, X. Chen, D. See and **J. Dubcovsky**. 2011. Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor Appl Genet. 123:143–157.

Cantu, D., M. Govindarajulu, A. Kozik, M. Wang, X. Chen, K.K. Kojima, J. Jurka, R.W. Michelmore and J. Dubcovsky. 2011. Next generation sequencing provides rapid access to the genome of *Puccinia striiformis* f.sp. *tritici*, the causal agent wheat stripe rust. *PloS ONE* 6:e24230

Presentations in scientific meetings:

Wu, K., D. Cantu, R. Ruan, A. Chen, D. Fu, P. Ronald, **J. Dubcovsky**. 2011. Wheat stripe rust resistance gene WKS1 can form homodimers that are important for interactions with downstream protein targets. Plant and Animal Genome XIX, January 15-19, San Diego, CA. P858

Lowe, I., L. Jankuloski1, S. Chao, X. Chen, D. See, **J. Dubcovsky**. 2011. Mapping and validation of *Yr48* and other QTL conferring partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Plant and Animal Genome XIX, January 15-19, San Diego, CA. P302.

Cantu D., , K. Wu, D. Fu, C. Uauy, A. Distelfeld, L. Epstein, P. Ronald, T. Fahima, J. Dubcovsky. 2010. What have we learned from the positional cloning of genes conferring partial resistance to wheat rusts? In Vitro Cellular and Developmental Biology. 46: S13

Lowe, I., L. Jankuloski, S. Chao, X. Chen, D. See, **J. Dubcovsky**. 2011. Discovery, mapping, and validation of QTL conferring partial resistance to broadly-virulent post-2000 North American races of stripe rust. BGRI Technical Workshop, St. Paul, MN June 13-16 2011.

Dubcovsky, J. 2011. Progress towards the positional cloning of *Sr13* and *Sr35.* BGRI Technical Workshop, St. Paul, MN June 13-16 2011.

Saintenac, C., W. Zhang, M. Rouse, E. Akhunov, **J. Dubcovsky**. 2011. Map based cloning and characterization of Ug99 resistance gene *Sr35*. 21st International Triticeae Mapping Initiative (ITMI), Mexico City, September 4 – 9, 2011.

Community Resources Generated:

TILLING mutants for RAR-1, XB12, VAP, WKS1

First *P. striiformis* genome publically available (http://maswheat.ucdavis.edu/plosonepst130/ http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0024230).

Other products/ outcomes:

Identification of *P. striiformis* proteins interacting with NPR1. This is an exciting result that opens a new area of research. We are currently trying to validate if this candidate effector competes with the previously identified NPR1-TGA interactions.

Training:

Postdocs: Dario Cantu (project direction, interactor characterization, PST genomics), Randi Ruan (Y2H library screening), Juan Sanchez (*in planta* validation of WKS1 interactors and phosphorylation targets of WKS1);

Visiting Scientist: Daolin Fu (validation of wheat-wheat interactions);

MS student: Kati Wu (characterization of WKS1 interactors, MS completed);

PhD students: Bajou Zhang (student from China, characterization of NPR1 interactors); Analía Espinoza (student from Chile *in planta* validation of WKS1 interactors).

Collaborations:

The sequencing of *Puccinia striiformis* race PST-130 has opened the door for an international collaboration on the sequencing and comparison of multiple PST races. International collaborators include Cristobal Uauy, John Innes Centre, Norwich, U.K, and Sophien Kamoun, The Sainsbury Laboratory, Norwich, U.K.

For the stem rust resistance gene *Sr35* work we established collaborations with Matt Rouse at the Cereal Disease laboratory and with Dr. Eduard Akhunov at Kansas State University.

For the stripe rust sequencing work we established collaborations with Dr. Richard Michelmore at UC Davis, with Dr. X Chen at the United States Department of Agriculture-Agriculture Research Service (USDA-ARS), Pullman, Washington, and Dr. J. Jurka at the Genetic Information Research Institute, Mountain View, California.

Triticeae CAP: Improving Barley and Wheat Germplasm for Changing Environments

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Project website: http://triticeaecap.org/

Objectives and Accomplishments:

***Genotyping*:** Illumina genotyping platforms including 9000 SNP markers have been used for genotyping of the NSGC barley core collection (2,446 accessions) and the wheat core collection (4,416 accessions). In addition, a set of 5,520 wheat breeding lines and mapping populations was genotyped with a 9000-SNP wheat assay (>100 million datapoints) developed in collaboration with the USDA wheat SNP project. More than 3,000 polymorphic SNPs have been integrated into both wheat and barley consensus genetic maps. Taken together, this extensive SNP information provides a detailed description of the genetic composition of the wheat and barley germplasm and is being used to identify associations between SNP markers and useful agronomic traits.

A successful pilot study for exon capture in wheat was completed with a 3.5 Mb capture array. The results were published in Genome Biology. Larger capture designs have been developed in barley (~60Mb) and wheat (~100Mb) as part of two international consortia in collaboration with Roche-Nimblegen. Hybridization tests will be initiated earlier next year. We also tested the approach for genotyping wheat and barley lines using a genotyping by sequencing approach and we are starting to use this technology to genotype diverse sets of lines and mapping populations. Two mapping populations have been genotyped by sequencing in wheat (ITMI mapping population) and barley (Oregon Wolfe Barley mapping population). In the wheat SynOpDH population we mapped over 27,000 SNP markers and 150,000 tags as dominant markers.

We have trained several different genomic selection models in barley for agronomic, disease, and grain quality traits. A manuscript comparing different GS models was published in Crop Science. For the winter barley GS experiments we completed 64 crosses using forty-seven facultative parents. The resulting F_1 and F_2 were grown in the 2011 greenhouse and in the field in St. Paul, MN, respectively. Phenotypes to train the GS model include freezing tests and winter survival in St. Paul, MN and are currently available for a total of 209 lines. Genomic breeding values will be used to select parents for the next round of crossing

The T3 database was established and the SNP and phenotypic data generated during the first year of the project are being entered into T3. We have currently entered 50.6 million genotypic data points and 100,105 phenotypic data points for multiple traits. A user group has been formed and has defined templates and pipelines to upload data to T3. This database is becoming a central hub for the US barley and wheat breeding programs.

***Phenotyping*:** To standardize the water and N use efficiency phenotyping using Canopy Spectral Reflectance (CSR), a CSR workshop was completed (40 participants). The first drought experiments confirmed the usefulness of CSR to detect differences in drought tolerance among wheat cultivars and isogenic lines. Near-isogenic lines for potential drought tolerance QTL have been investigated in rainfed and irrigated environments. Agronomic and physiological traits and high-throughput canopy spectral reflectance indices were evaluated in 540 accessions from the wheat NSGC core collection. QTL associated with water and nitrogen use efficiency are being identified.

One thousand lines of the wheat NSGC core collections were evaluated for stripe rust in CA and WA (data has been entered into T3) and for leaf rust and stem rust in MN. New genes for resistance to stripe rust have been identified and published. A high-density map of the *Yr48* resistance gene was completed and the positional cloning of this gene was initiated. A thousand barley lines were also evaluated for resistance to spot blotch. The resistant lines are being incorporated into breeding program crossing blocks. Screening of ~1000 lines from the barley NSGC core for the spot form of net blotch was completed and data is being analyzed.

The research activities resulted in 25 manuscripts accepted for publication in peer reviewed journals, numerous presentations in scientific meetings and the release of 14 new varieties and 12 improved germplasm.

***Education*:** A total of 94 four students and plant breeding professionals have participated in T-CAP training first year activities. Twenty three PhD students have initiated their training programs on T-CAP research projects (eleven above year one target by leveraging funds). The Plant Breeding Training Network has been launched and is being used by undergraduates, graduate students and PIs. Over 30 graduate students have been meeting regularly to help test the PBTN, and have begun to build community and share ideas. A graduate level course for 21 students has been offered online. Four other graduate students are participating in the project through evaluation or film production. Fourteen undergraduates have begun work with TCAP researchers and met online with a representative from Pioneer.

The Education team and evaluators had a successful meeting with representatives from Minority Serving Institutions (MSI) to establish collaborations. MSI recommendations made through a focus group were implemented in the creation of a request for proposals (RFP). The RFP was distributed to about 80 MSIs and we received 12 proposals that were evaluated and 8 were awarded (total awards $80,000). MSI and TCAP faculty are building collaborative relationships and seven students at MSIs have begun work.

The education team organized a successful launching meeting in San Diego, an online meeting with the advisory panel, a Canopy Spectral Reflectance training in Denver, developed educational and evaluation tools and published two newsletters. The education team also hosted a talk for breeding for climate change at the National Association for Plant Breeders and supported attendance of over 70 students from around the country.

Broad Impacts:

The T-CAP helped US wheat and barley breeders to accelerate the release of 14 new varieties, 12 new improved germplasm and two recombinant-inbreed mapping populations. In addition, tens of thousands of barley and wheat breeding lines were selected with molecular markers and are now at different levels of testing in breeding program pipelines. Therefore, the positive impact of the T-CAP is now affecting a large proportion of the barley and wheat breeding programs. This project has empowered public breeders to accelerate the incorporation of valuable genes into barley and wheat varieties using modern molecular technologies. The project also initiated the testing of genomic selection methods in barley and wheat. By allowing selection in each crossing cycle, genomic selection is expected to greatly accelerate the rate of improvement for targeted traits.

The T-CAP contributed to the first genomic sequence of *Puccinia striiformis,* the causal agent of stripe rust. The availability of this sequence has allowed the identification of ~1000 candidate effectors, which are the proteins pathogens use to control the host cells. The publication of this information has triggered multiple studies to validate these effectors and provided multiple potential targets to fight this devastating pathogen.

The project also published molecular markers for several new stripe rust resistance genes in wheat (see publications below). These markers facilitate the engineering of varieties with multiple resistance genes, a strategy that has been successful to control the stripe rust epidemic in the western US. The deployment of resistant varieties reduces the use of pesticides, minimizing their negative impact on the environment and on production costs. During this first year of the project, molecular markers have been also published for disease resistance genes (soil-borne wheat mosaic virus, fusarium head blight, tan spot) and agronomic traits (number of productive tillers, drought tolerance).

In the quality area, studies were published for markers for grain protein content, flour color, milling yield, endosperm texture, dough-mixing strength, and bread-making properties. The incorporation of genes with favorable effects on quality contributed to increased value of the barley and wheat crops and the competitiveness of the US growers. Several of these favorable alleles have been already incorporated into commercial public varieties released this year.

One of the major accomplishments of the T-CAP during its first year has been the completion of the genotyping of the core barley and wheat collections from the NSGC with a 9000 SNP platform. These data provide a comprehensive characterization of the genetic diversity of the US wheat and barley germplasm collections, a platform for association mapping studies, and the opportunity to select parental lines for developing nested association mapping populations. The phenotypic characterization of the core collections for disease resistance, water use efficiency and nitrogen use efficiency was initiated and the year one datasets are being analyzed for associations between genotypic and phenotypic traits. These associations are the basis for the identification of valuable alleles that will then be used by the breeding programs to add value to their lines.

The T-CAP was instrumental in attracting new PhD students to plant breeding. In addition, the organization of a centralized training network has provided students in different parts of the country access to experts in the different scientific disciplines that support plant breeding. The T-CAP educational web site was also used to deliver educational and outreach materials. In summary, the T-CAP has generated an integrated network of public wheat breeding programs, and promoted collaboration among the US barley and wheat breeding and genotyping programs accelerating the development of improved varieties for the different cereal growing regions of the US. It has also empowered the US breeding programs to engage in large international efforts aimed to accelerate the improvement of these two important crops.

Deliverables:

Publications:

Accepted and in press in peer reviewed journals (25)

1.- Lowe, I., D. L. Jankuloski, **S. Chao**, **X. Chen**, **D. See** and **J. Dubcovsky**. 2011. Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor Appl Genet. 123:143–157.

2.- Chen, J., Ch. Chu, **E.J. Souza**, **M.J. Guttieri**, **X. Chen**, S. Xu, **D. Hole**, and R. Zemetra. 2011. Whole genome-wide mapping for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp*. tritici*) in a hard red winter wheat germplasm IDO444. Molecular Breeding (DOI 10.1007/s11032-011-9590-x).

3.- Zhang, D., **G. Bai**, R. M. Hunger, W. W. Bockus, J. Yu, **B. F. Carver**, and **G. Brown-Guedira**. 2011. Association study of resistance to *Soilborne Wheat Mosaic Virus* (SBWMV) in U.S. winter wheat. Phytopathology 101:1322-1329.

4.- Bernardo A. N., H. Ma, D. Zhang, and **G. Bai**. 2011. Single Nucleotide Polymorphism in Wheat Chromosome Region Harboring *Fhb1* for Fusarium Head Blight Resistance. Mol Breed. DOI 10.1007/s11032-011-9565-y.

5.- Tsilo, T., G.A. Hareland, **S. Chao**, and **J.A. Anderson**. 2011. Genetic mapping and QTL analysis of flour color and milling yield related traits using recombinant inbred lines in hard red spring wheat. Crop Sci. 51:237-246.

6.- Tsilo, T.J., G.L. Linkert, G,A. Hareland, and **J.A. Anderson**. 2011. Registration of the MN98550–5/MN99394–1 wheat recombinant inbred mapping population. J. Plant Registrations 5: 257–260.

7.- Tsilo, T.J., S. Simsek, J.-B. Ohm, G.A. Hareland, **S. Chao**, and **J.A. Anderson**. 2011. Quantitative trait loci influencing endosperm texture, dough-mixing strength, and bread-making properties of the hard red spring wheat breeding lines. Genome 54: 460-470.

8.- Saintenac, C., D. Jiang, **E. Akhunov**. Targeted analysis of nucleotide and copy number variation by exon capture in allotetraploid wheat genome. Genome Biology, 12:R88.

9.- Cantu, D., M. Govindarajulu, A. Kozik, M. Wang, X. Chen, K. Kojima, J. Jurka, R.W. Michelmore, and **J. Dubcovsky**. 2011. Next generation sequencing provides rapid access to the genome of *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust. PlosOne. 6(8): e24230. doi:10.1371/journal.pone.0024230.

10.- Naruoka, Y., **L. E. Talbert**, S. P. Lanning, N. K. Blake, J. M. Martin and **J. D. Sherman**. 2011. Genetics of productive tiller number and its relationship to economic traits in spring wheat. Theor. Appl. Genet. 123:1043-1053.

11.- Kalous, J. R.**,** J. M. Martin, **J. D. Sherman**, N. K. Blake, S. P. Lanning and **L. E. Talbert**. 2011. Phenotypic variation and patterns of linkage disequilibrium associated with introduced genes in spring wheat. Crop Sci. 51: 2466-2478.

12.**-** Blake, N.K., S.P. Lanning, J. E. Berg, P. L. Bruckner, **J. D. Sherman** and **L.E. Talbert**. 2011. Registration of spring and winter habit wheat lines derived from elite cultivars of the alternate growth habit. J. Plant Reg. 5:418-421.

13.- Li, P., **J. Chen**, P. Wu, J. Zhang, Ch. Chu, **D. See**, **G. Brown-Guedira**, R. Zemetra, and E. Souza. 2011. QTL analysis for the effect of *RhtB1* dwarfing gene on coleoptiles length, seedling root length and numbers of bread wheat (*Triticum aestivum* L.). Crop Sci. 51: 2561-2568.

14.- Noriel, A.J., X.-C. Sun, W. Bockus and **G.-H. Bai**. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. Crop Sci. 51:1059-1067

15.- Wang, H., **K.P. Smith**, E. Combs, **T. Blake, R. Horsley,** and **G.J. Muehlbauer.** 2011. Effect of population size and unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. Theor. Appl. Genet. DOI 10.1007/s00122-011-1691-8.

16.- **Prasad, P.V.V**., S.R. Pisipati, I. Momcilovic, and Z. Ristic. 2011. Independent and combined effects of high temperature and drought stress during grain filling and plant yield and chloroplast EF-Tu expression in spring wheat. Journal of Agronomy and Crop Science 197: 430-441.

17.- Pradhan G.P., **P.V.V. Prasad**, A.K. Fritz, M.B. Kirkham, and **B.S. Gill**. 2011. High temperature tolerance in Aegilops species and its potential transfer to wheat. Crop Science *In press*.

18.- Heslot, N., H.-P. Yang, **M.E. Sorrells**, and **J-L. Jannink**. 2011. Genomic selection in plant breeding: A comparison of models. Crop Science. Accepted with minor revisions.

19.- Naruoka, Y., **J. D. Sherman**, S. P. Lanning, N. K. Blake, J. M. Martin, and **L. E. Talbert**. 2012. Genetic analysis of long green leaf duration in spring wheat. Crop Sci. *In Press*. DOI:10.2135/cropsci2011.05.0269.

20.- Hao, Y., Z.Chen, Y.Wang, D. Bland, J. Buck, **G. Brown-Guedira** and J. Johnson. 2011. Characterization of a novel major QTL for adult plant resistance to stripe rust in US soft red winter wheat. Theor. Appl. Genet. *In press.*

21.- Iwata, H. and **J.-L. Jannink**, 2011. Accuracy of genomic selection in barley breeding programs: a simulation study based on the real SNP data. Crop Sci. 51:1915-1927.

22.- Li. P., **J. Chen**, and Pute Wu. 2011. Evaluation of Grain Yield and Three Physiological Traits in 30 Spring Wheat Genotypes across Three Irrigation Regimes. Crop Sci. 52:1-12.

23.- Pradhan G.P., **P.V.V. Prasad**, A.K. Fritz, M.B. Kirkham, and **B.S. Gill**. 2011. Response of Aegilops species to drought stress during reproductive stages of development. Functional Plant Biology. *In press.*

24.- Edwards, J.T., R.M. Hunger, E.L. Smith, G.W. Horn, M.-S. Chen, **L. Yan**, **G. Bai**, **R.L. Bowden**, **A.R. Klatt**, P. Rayas-Duarte, R.A. Osburn, **J.A. Kolmer**, **Y. Jin**, D.R. Porter, K.L. Giles, B.W. Seabourn, M.B. Bayles, and **B.F. Carver**. 2012. 'Duster' wheat: A durable, dual-purpose cultivar adapted to the southern Great Plains of the USA. J. Plant Reg. 6:1-12.

25.- **Morrell, P.L.**, Buckler, E.S., Ross-Ibarra, J. in press. Crop genomes: advances and applications. Nature Reviews Genetics

***Articles in Proceedings*: E. Akhunov**, **S. Chao**, V. Catana, **D. See**, **G. Brown-Guedira**, **M. Sorrells**, A. Akhunova, **J. Dubcovsky**, C. Cavanagh and M. Hayden. New tools for wheat genetics and breeding: genome-wide analysis of SNP variation. Proceedings of BGRI Technical Workshop, June 13-16, 2011, St. Paul, Minnesota, U.S.A.

***Presentations in meetings and scientific symposia*:** 22 presentations were made

***New Germplasm*:** The new released varieties include four barley and ten wheat lines (two pasta and eight bread-wheat varieties). The development of these varieties was initiated in the Barley and Wheat CAP projects and completed under the T-CAP project. The four barley varieties released in 2011 were Atlantic, Voyager, Transit and Verdant. The high quality durum wheat varieties PVP in 2011 "Desert King-High Protein" and "Tipai" were selected with molecular markers for improved grain protein content (*GPC-B1*), improved semolina color (*PSY1* A-B hybrid allele) and higher pasta color stability (lipoxygenase deletion).

Three common soft and hard winter wheat cultivars from ID are being released based on their yield potential under water limited conditions (SWS IDO599, SWS IDO671, & HWS IDO694). The solid-stem variety 'SY Tyra' (resistant to wheat stem sawfly) was developed by marker assisted backcrossing in collaboration between Montana State University and Syngenta and is being marketed in Montana and North Dakota. The dual-purpose variety 'Duster' was released by the Oklahoma State University and is well adapted to the southern Great Plains of the USA.CSU released three new varieties: Byrd, Denali, and Brawl CL Plus. Byrd is a high yielding variety under both dryland and irrigated conditions, carries

stripe rust resistance and an unknown source of Ug-99 stem rust resistance, and has excellent milling and baking quality. Denali has shown excellent yields across the High Plains. Brawl CL Plus is the first public two-gene Clearfield wheat, showing comparable yields as other Colorado Clearfield varieties in addition to improved stripe rust resistance, test weight, and milling and baking quality.

In addition to finished varieties, molecular markers were used to release improved germplasm, including 6 hard red spring wheat donor lines with new stripe rust resistance gene combinations (Lowe et al. 2011, TAG. 123:143–157, GSTR13606 and GSTR 13634, GSTR13600, GSTR13664, GSTR13504, GSTR13618). Molecular markers were also used to develop 6 isogenic lines of hexaploid wheat for vernalization alleles to provide wheat breeding programs access to genes from elite cultivars possessing alternate growth habits (Blake et al. 2011, JPR 5: 418-421, PI 660648, PI 660649, PI 660650, PI 660651, PI 660652, PI 660647**)**

Two new recombinant inbred mapping populations were deposited in the NSGC: MN98550 x 5/MN99394–1 (139 lines adapted to the Upper Midwest region of the USA) and UC1110 x PI610750 (186 lines GSTR numbers 13501-13687 with new sources of stripe rust resistance)

Community Resources Generated:

9000 SNP Illumina platforms for barley and wheat, available to breeders for rapid genotypic characterization of their lines and mapping populations.

High density SNP maps of barley and bread wheat.

The genomic sequence of stripe rust and a list of potential effectors.

T3 database for wheat and barley breeders and geneticists.

Other products/ outcomes:

Plant Breeding Training Network

http://passel.unl.edu/communities/index.php?idcollectionmodule=1130274157

Protocols for MAS for more than 60 traits

http://maswheat.ucdavis.edu/protocols/index.htm

Two issues of the T-CAP newsletter have been produced

Training:

A total of 94 professionals and students have benefited from TCAP education activities. Twenty three PhD students (eleven above first year target) have initiated their training programs during the first year of the T-CAP (Bajgain, Prabin; Bowman, Brian; Cobo, Nicolas; Falcon, Celeste; Fang, Tilin; Felipe Salcedo Jordán, Andrés; Frels, Katherine; Godoy, Jayfred; Grogan, Sarah; Hazard, Brittany; Hegarty, Josh; Hoffstetter, Amber; Howell, Tyson; Kalous, Jay; Mahmoud, Wahid; Merrill, Keith; Narayanan, Sruthi ; Nice, Liana; Nitcher, Rebecca; Pauli, William (Duke); Seda, Brian ; Turner, Kathryn; and Zhang, Junli). Fourteen of these students have already been paired with undergraduate students to develop their mentorship skills. In addition two film students and two education students participate in the project.

The Plant Breeding Training Network has been launched and is being used (http://passel.unl.edu/pagespbtn/). Thirty two graduate students met regularly over the summer to help test the PBTN, and in the process have begun to build community and share ideas. Twenty one students are participating in the fall online class "Plant Breeding Strategies" taught by Jamie Sherman with the support of TCAP PIs through archived lectures.

The education team organized a successful launching meeting in San Diego, an online meeting with the advisory panel, a Canopy Spectral Reflectance training in Denver (42 participants), developed educational and evaluation tools and published articles in two newsletters. The education team also hosted a talk for Breeding for Climate Change at the National Association for Plant Breeders and supported attendance of over 70 students from around the country.

The Education team and evaluators had a successful meeting with representatives from Minority Serving Institutions (MSI) to establish collaborations. MSI recommendations made through a focus group were implemented in the creation of a request for proposals (RFP). The RFP was distributed to about 80 MSIs and 8 proposals were funded at the requested level ($80,000). Funded MSI faculty members are from Chicago State University, Tuskegee University, Texas A&M (Amarillo), University of Arkansas (Pine Bluff, two proposals), Lehman College (New York), Zhu - Rust College, and Fayetteville State University. Successful MSI faculty members were paired with T-CAP faculty members and collaborative relationships are being built and seven MSI undergraduate students have started research projects. Mary Brakke hosted all undergraduates in an online meeting with a representative from Pioneer.

The following evaluation Tools have been developed and implemented: 1) TCAP and MSI faculty were surveyed to determine the need for educational tools; 2) TCAP faculty and students were surveyed to determine the baseline data that will be used to assess change over the life of grant; 3) A student evaluator will participate in online meetings using a rubric created to assess effectiveness; 4) An interview tool was developed to assess faculty and student perceptions of educational activities.

Collaborations:

The T-CAP has catalyzed multiple interactions among breeding programs, genotyping labs and barley and wheat researchers that would have not been possible without the T-CAP support. The T-CAP has promoted collaboration among the US breeding and genotyping programs accelerating the development of improved wheat and barley varieties. The T-CAP has also provided the necessary support to engage in large International collaborations such as the development of the barley 9000 SNP platform, the barley and wheat gene capture Nimblegen platforms, the construction of high-density SNP consensus maps and the initiation of compatible databases among large projects in CIMMYT, Europe, Australia, Canada and the US.

T-CAP initiated research collaborations with eight minority serving institutions (see above). T-CAP members S. Baenziger, D. Namuth-Covert, and J. Sherman with private plant breeders and industry professional are forming a consortium to provide online course sharing for plant breeding students. T-CAP is providing support to identify and overcome barriers in course sharing as well as material support by providing an online meeting environment.

Simultaneous Genetic Analysis of Winter Hardiness Traits and Development of Winter Malting Barley Varieties

Patrick Hayes

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Co-PDs: S. Baenziger, UNL- USA; Z. Bedo, MRI-HUN; B. Cooper, LG-USA; L. Cistue, AD-ESP;C. Henson and M. Schmitt, USDA-ARS-WI-USA; and R. Waugh, JHI-SCT.

Project website: barleyworld.org

Objectives and Accomplishments:

This project is designed to increase the profitability and sustainability of US agriculture by developing winter malting barley varieties with superior cold tolerance and quality. Fall-sown barley will be increasingly important in the era of climate change due to higher yield potential and efficient use of water resources. Resistance/tolerance to biotic and abiotic stresses will be critical. Low temperature tolerance (LTT) is a biotic stress of great importance. Resistance to barley stripe rust (incited by *Puccinia striifomis* f. sp. *hordei)* and scald (incited by *Rhynchosporium secalis*) will be important in higher rainfall areas. Simultaneous gene discovery and breeding will accelerate the development of agronomically relevant germplasm.

We developed two doubled haploid mapping populations using two lines from the University of Nebraska (NE) with superior cold tolerance and one line from Oregon State University (OR) with good malting quality and disease resistance: NB3437f/OR71 (facultative x facultative) and NB713/OR71 (winter x facultative). Both were genotyped with a custom 384 oligonucleotide pool assay (OPA). QTL analyses were performed for low temperature tolerance (LTT), vernalization sensitivity (VS), and resistance to barley stripe rust and scald. Disease resistance QTL were identified with favorable alleles from both NE and OR germplasm. The role of *VRN-H2* in VS was confirmed and a novel alternative winter allele at *VRN-H3* was discovered in the Nebraska germplasm. *FR-H2* was identified as a determinant of LTT and a new QTL, *FR-H3*, was discovered on chromosome 1H that accounted for up to 48% of the phenotypic variation in field survival at St. Paul, Minnesota, USA. The discovery of *FR-H3* is a significant advancement in barley LTT genetics and will assist in developing the next generation of fall-sown varieties. Other aspects of this project are ongoing as planned.

Broad Impacts:

More cold tolerant barley with malting quality will offer new economic options for farmers, processors, and consumers. The discovery of *FR-H3* is an exciting target for further breeding and science research. This exploration may lead to the discovery of a mechanism for achieving higher levels of cold tolerance in barley.

Deliverables:

Publications:

Submission of the mapping and QTL reports in December, 2011.

Oral/ Poster Presentations:

Fisk, S., S. Baenziger, Z. Bedo, L. Cistue, A. Corey, Y. Chutimanitsakun, A. Cuesta-Marcos, B. Cooper, T. Filichkin, N. Graham, P. Hayes, C. Henson, M. Schmitt, and R. Waugh. Simultaneous genetic analysis of winter hardiness traits and development of winter malting barley varieties. Poster session presented at: The Plant and Animal Genome Conference XVIII. 2010 Jan 9-13; San Diego, CA.

Fisk, S., S. Baenziger, Z. Bedo, L. Cistue, A. Corey, Y. Chutimanitsakun, A. Cuesta-Marcos, B. Cooper, T. Filichkin, N. Graham, P. Hayes, C. Henson, M. Schmitt, and R. Waugh. Simultaneous genetic analysis of winter hardiness traits and development of winter malting barley varieties. Poster session presented at the NIFA PI meeting. Jan 13; San Diego, CA.

Community Resources Generated:

Two doubled haploid populations, available upon request from the Project Director. All genotype and phenotype data will be made available at the time of publication.

Other products/ outcomes:

The 384 custom OPA is available.

Training:

S. Fisk, Y. Chutimanitsakun, and N. Graham, graduate students. A. Cuesta-Marcos, post-doc; A. Corey and T. Filichkin, technicians. Participated in generating and/or analyzing data culminating in 2010 PAG poster, the 2011 NIFA PI poster, this report, and the forthcoming publications.

Collaborations: New partnerships may be developed in order to explore *FR-H3*.

Oat SNP Development and Identification of Loci Affecting Key Traits in North American Oat Germplasm Using Association Genetics

Eric Jackson

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Project website: www.ars.usda.gov/oat

Objectives and Accomplishments:

Objective 1. Develop genetic marker resources for MAB of key oat traits: 2,183 new oat-based SNP markers have been validated across 113 varieties, five mapping populations, and a series of chromosome deficient anuepliod hybrids. The genotyping results were used to create the first physically anchored oat consensus map, the most significant impact in oat research over the last 30 years (*See manuscript submission below*). A new SNP genotyping array has been developed and used to genotype elite lines from over 10 oat breeding programs world-wide. A genotype-by-sequencing (GBS) methodology is currently being validated for oat and will be used to genotype the entire oat population by mid-to-late spring, 2012.

Objective 2. Conduct a two year multi-location association mapping study to develop marker-trait associations: Three oat association mapping populations consisting of 685 lines have been evaluated in 17 locations across the globe. Agronomic and disease data from various locations in both years have been collected and uploaded into The Avena Toolbox (TAT). Barley yellow dwarf virus (BYDV) and crown rust data has been used to localize QTL affecting each trait. Comparative genomics studies have identify candidate genes for each QTL. Milling data has been collected and uploaded for the 2011 field trials and analyses of beta glucan, total dietary fiber, tocopherol, tocotrienol, and avenanthramide content are being finalized.

Objective 3: Develop online portal for utilization and dissemination of project information: Genotypic and phenotypic data from 2011 has been up loaded to TAT and is available http://avena.pw.usda.gov/tat. Additionally, a new BLAST server is available in TAT. Complimentary to the TAT, a new Breeding Oat Application Tool or BOAT is being developed. The application will be accessible from smart phones or common laptop computers by mid-summer 2012.

Objective 4. Functional marker development: One hundred and fifty four candidate predictive SNP assays have been validated for 13 triats across 288 oat lines from key North American breeding programs. From this work, predictive assays for partial crown rust and barley yellow dwarf resistance, oil, beta glucan, vitamin E and various agronomic traits are now available.

Broad Impacts:

Several unanticipated outcomes have been realized and achieved through this project including the development of genetic linkage mapping, association mapping, and gene expression tools within the JMP Genomics 5.1 software (SAS Institute, Cary, NC). We have also made considerable progress on the genome relationships in regards to expression of key traits like beta glucan and unsaturated fats. We have developed new techniques to utilized gene-based markers in QTL analysis to quickly identify candidate genes using comparative genomics and screen large populations of lines for specific functional mutations resulting in a phenotype. We have also developed two new SNP selection approaches for complex genomes that will benefit orphan crops with complex genomes.

Deliverables:

Publications:

R. E. Oliver, G. R. Lazo, J. D. Lutz, M. J. Rubenfield, N. A. Tinker, J. M. Anderson, E. W. Jackson, et al. *2011*. Model SNP Development for Complex Genomes Based on Hexaploid Oat Using High-Throughput 454 Sequencing Technology. *BMC Genomics 12:77*.

Rebekah E Oliver, Eric N Jellen, Gideon Ladizinsky, Abraham B Korol, Andrzej Kilian, Eric W Jackson, et al. *2011*. New Diversity Arrays Technology (DArT) markers for tetraploid oat (Avena magna Murphy et Terrell) provide the first complete linkage map and markers linked to domestication genes from hexaploid *A. sativa* L. *Theoretical and Applied Genetics 7:1159-1171*.

R.E. Oliver, S. Chao, G.R. Lazo, N.A. Tinker, E.N. Jellen, M.L. Carson, H.W. Rines, E.W. Jackson, et al. *Submitted*. Two decades later: Novel SNP identification and chromosome-deficient hybrid anchoring strategies provide the first physically-anchored integrated map of the complex oat genome. *PNAS*.

R.R. Redman, E.N. Jellen, R.E. Oliver, P.J. Maughan, G.R. Lazo, D. Adhikary, S.E. Oliver, J.L. Beard, and E.W. Jackson. *Submitted*. New tetraploid oat SNP markers provide a methodology for genomic SNP development in complex genomes and shed light on the ancestral mystery of oat. *Theoretical and Applied Genetics*.

R.R. Redman, E.N. Jellen, R.E. Oliver, P.J. Maughan, J.L. Beard, and E.W. Jackson. *Submitted*. Use of tetraploid oat-based SNPs to update the existing genetic linkage map and study the diversity of tetraploid Avena accessions in the National Small Grains Collection. *BMC Genetics*.

G.R. Lazo, R.E. Oliver, N.A. Tinker, J.D. Lutz, E. Islamovic, E.W. Jackson, et al. *Submitted*. Oat transcriptome sequencing reveals intra- and intergenomic relationships: A platform for gene discovery. *PNAS*.

Oral/ Poster Presentations:

Oral presentation to Southern Wheat Workers (*Jackson*); Oral presentations at the Plant and Animal Genome (*Jackson, Polland, and Chao*); Poster presentation at the Plant and Animal Genome (*Sheridan*); Oral presentations at the 2011 CORE Workshop in Dallas, TX (*Jackson, Tinker, Chao, Lazo*) ; Oral presentation at the North American Millers Association Meeting in Marco Island, FL (*Jackson*).

Community Resources Generated:

The following high impact items have been produced: 1) Linkage and association mapping in JMP Genomics 5.1, 2) 2,183 new oat SNP markers, 3) the first complete (21 linkage group) physically anchored hexaploid oat map and corresponding populations, 4) Functional TAT database with oat BLAST engine, and 5) Validated predictive assays for partial crown rust QTL.

Training:

Students:

Rebekah Oliver, Postdoctoral Fellow ARS – Marker development and population development; Aberdeen, Emir Islamovic, Postdoctoral Fellow ARS Aberdeen – Gene discovery; Rachel Redman, Masters Student Brigham Young University – Marker development; Mellissa Coon, Masters Student Brigham Young University – Gene discovery; Carol Lang, Masters Student Texas A & M University – Crown and stem rust mapping; Bradley Foresman, Masters Student University of Illinois – BYDV mapping; Scott Smith, Masters Student Brigham Young University – Gene discovery and fiber mapping.

Workshops:

Training Workshop for Oat Researchers: Linkage Mapping and Association Mapping using JMP Genomics Software – College Station, TX October 25-27, 2011.

Training Workshop for Rice Researchers: Linkage Mapping and Association Mapping using JMP Genomics Software – Stuttgart, AR November 29-December 1, 2011.

Collaborations:

Collaborations have now been formed with the Norwegian University of Life Sciences, Norway; The Federal University of Rio Grande, Brazil; SARDI and the National Oat Breeding Program, Australia; Agricultural University, Lublin, Poland; and JMP Genomics a Division of the SAS Institute Inc. Cary, NC.

A Genome Sequence for Common Bean, Phaseolus vulgaris

Scott Jackson

Project Director: Scott Jackson, sjackson@uga.edu

Co-PDs: Jeremy Schmutz, jschmutz@hudsonalpha.org; Phil McClean, phillip.mcclean@ndsu.nodak.edu; Perry Cregan , creganp@ba.ars.usda.gov

Project website: www.phytozome.org

Objectives and Accomplishments:

The goal is to provide an integrated genome sequence for common bean (Phaseolus vulgaris) for the community to use for improvement of common bean and to investigate genome structure and evolution in the Phaseoloid legumes.

- Sequencing: a whole genome sequence consisting of 454, Illumina and Sanger sequencing that captures more than 80% of the genome in sequence contigs/scaffolds.
- Annotation of the genome sequence.
- Development, mapping and release of >6,000 SNP markers based on genome sequence contigs.

Broad Impacts:

The use of the genome sequence and genetic markers for crop improvement. Use of the genome sequence to help guide the assembly of an international effort to sequence a MesoAmerican genotype.

Deliverables:

Genome sequence and annotation, phytozome.org

>6,000 SNP markers, (Perry Cregan, to be published)

Publications:

Varshney, R.K., W. Chen, Y. Li, A.K. Bharti, RK Saxena, JA Schlueter, MT.A. Donoghue, S. Azam, G. Fan, A.M. Whaley, A. Farmer, R. Tuetja, R.V. Penmetsa, W. Wu, H. Upadhyaya, S-P. Yang, T. Shah, K.B. Saxena, E. Ward, T. Michael, W. R. McCombie, B. Yang, J.D.G. Jones, C. Spillane, D.R. Cook, G.D. May, X. Xu, S.A. Jackson. 2011. Genome sequence for pigeonpea. Nature Biotech. doi:10.1038/nbt.2022

Jackson, S.A. A. Iwata, S-H Lee, J. Schmutz and R.C. Shoemaker. 2011. Sequencing Crop Genomes: Approaches and Applications. New Phyt. doi: 10.1111/j.1469-8137.2011.03804.x.

Cordoba, J.M., M.C. Chavarro, J.J. Schlueter, S.A. Jackson and M. Blair. 2010. Integration of physical and genetic maps of common bean through microsatellite markers. BMC Genomics 2010, 11:436.

Oral/ Poster Presentations:

Lin, J-Y., R.M. Stupar, C. Hans, D. Hyten and S.A. Jackson. 2010. Comparative analysis of a 1-mb region of *Phaseolus vulgaris* to the highly duplicated soybea genome. Plant and Animal Genome XVIII. San Diego, CA.

Stupar, R.M., J-Y. Lin, S.A. Jackson, Y-T. Bolon, G. Muehlbauer, S. Naeve, J. Orf, C. Vance, W.J. Haun. 2009. Transcriptional profiling and mutational detection of soybean homeologous genes. World Soybean Research Congress. Beijing, China.

Genomics in legumes. 2011. Seoul National University. Seoul, South Korea.

Genome diversity and evolution. 2011. 6th US-Mexico Plant Biology and Biochemistry Conference. Campeche, Mexico.

Genome sequencing of Phaseolus vulgaris. 2011. Phaseolus Genomics Conference. Barcelona, Spain.

Genomics for genome evolution and crop improvement in Legumes. OzBio 2010: The molecules of life-from discovery to biotechnology. Melbourne, Australia.

Legume genomics for developing countries. 2011. ADNAP. Hyderabad, India.

Genomics to improve legumes, here and around the world. 2010. Danforth Center for Plant Sciences. St. Louis, MO.

Next Generation sequencing in crop legumes: a focus on developing countries. 2010. CSSA meetings. Long Beach, CA.

Recurrent polyploidy in legumes. 2010. Plant Biology Department. University of Minnesota. St. Paul, MN.

Sequencing plant genomes. 2010. HudsonAlpha Institute for Biotechnology, University of Alabama, Huntsville, AL.

Using genomics for soybean improvement. 2010. DC Smith Memorial Lectureship. University of Wisconsin-Madison, WI.

Agricultural Genomics. 2010. Agricultural College of the Armed Forces. West Lafayette, IN.

Community Resources Generated:

Genome sequence for Phaseolus available at phytozome.org

Training: Mirayada Torres-Torres, PhD student.

Collaborations: JGI/DOE; Spanish-Mexican-Brazilian Phaseolus genome sequencing project.

Common Bean Coordinated Agricultural Project (CAP)

Phil McClean

Project Director: Phil McClean, North Dakota State University

Co-PDs: Julie Garden-Robinson, North Dakota State University, julie.garden-robinson@ndsu.edu; Paul Gepts, University of California, Davis, plgepts@ucdavis.edu; Michael A. Grusak, USDA-ARS Children's Research Center, mgrusak@bcm.tmc.edu; James D. Kelly, Michigan State University, kellyj@msu.edu; Phillip N. Miklas, USDA-ARS, Vegetable and Forage Crop Production Research Unit, Phil.Miklas@ARS.USDA.GOV; Jim R. Myers, Oregon State University, myersja@hort.oregonstate.edu; Juan M. Osorno, North Dakota State University, juan.osorno@ndsu.edu; Mark A. Brick, Colorado State University, mark.brick@colostate.edu Karen A. Cichy, USDA-ARS Sugar Beet and Bean Research Unit, Karen.Cichy@ARS.USDA.GOV; Perry B. Cregan, USDA-ARS Soybean Genomics and Improvement Laboratory, Perry.Cregan@ARS.USDA.GOV; Christina Johnson, North Dakota State University, christina.johnson@ndsu.edu; Timothy Porch, USDA-ARS Tropical Agriculture Research Station, timothy.porch@ars.usda.gov; Elizabeth Ryan, Colorado State University, e.p.ryan@colostate.edu; Henry J. Thompson, Colorado State University, henry.thompson@colostate.edu; Carlos A. Urrea, University of Nebraska-Lincoln, currea2@unlnotes.unl.edu

Project website: http://www.beancap.org

Objectives and Accomplishments:

Objective 1: Marker development. Within and between market class SNPs were discovered using Illumina GAII generated sequence data for eighteen genotypes representing the pinto, navy, black, Great Northern, and kidney market classes. 1,859,249 SNPs were discovered. The SNP data was used to develop a 6000 SNP Illumina Infinium SNP detection chip that was used to develop a ~2600 SNP map and evaluate sequence variation with the major market classes. The genotype sequence data was mined for indels >7nt, and the information was used to develop ~2000 indel markers for both within and between market class genotyping. ***Objective 2: Nutritional analysis and field trial.*** Greenhouse grown exhibited at least a 2X difference for 11 minerals. Within market class differences were detected for most minerals. Association mapping using the SNP and nutritional data discovered 8 loci significantly associated with iron content in the seed, one of which maps within 110kb (less than the LD distance) of a gene known to be involved in Fe metabolism. Field trials of 300 Mesoamerican cultivars were established in CO, MI, ND, and NE. ***Objective 3: Database.*** The PhaseolusGenes

(http://phaseolusgenes.bioinformatics.ucdavis.edu/search/) database was populated with 795 SSR, 633 STS, 76 SCAR, and 38 gene-based markers. 21,825 new SSRs were discovered using 1x methyl-filtrated sequence data and mapped relative to the soybean genome. 888 QTL were identified from literature searches and entered into the database. A GBrowse version of the database was developed to display the marker data. ***Objective 4: Outreach.*** The "Now Serving: Beans!" teaching kit for teens and adults that provides nutritional facts about beans and ideas for adding them to menus was beta tested with 150 Family and Consumer Science teachers and Extension agents and released. Two bean recipe videos were released on the BeanCAP YouTube channel (http://www.youtube.com/user/ndsubeancap). A 4:22 minute video describing the role of plant breeding in food security was developed and released on the channel. The animation team completed the first pair of modules that focus on 1) root biology and the role soil chemistry plays on nutrient uptake; and 2) the flow of soil minerals from the root to various parts of plants. ***Objective 5: Plant breeding education.*** The ongoing hands-on internships occurring at four institutions (CSU, MSU, NDSU, and UNL) have proven to be the most successful way to spark interest in the students exposed. A total of 18 students (high school and undergraduates) across all institutions on the everyday activities of a breeding program with the goal of letting the students learn by practical experiences and not just in a passive manner. High school visits emphasized the role of plant breeding in the food system and plant breeding as a career. Presentations at national meetings focused on the unique aspect of the project and served as a recruitment vehicle. Two BeanCAP intern alumni entered graduate school in plant breeding.

Deliverables

Publications:

McClean, P.E., Burridge, J., Beebe, S., Rao, I.M., Porch, T.G. 2011 Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean (*Phaseolus vulgaris*) Functional Plant Biology (published on-line November 8, 2011).

Mamid, S., Rossi, M., Annam, D. Moghaddam, S., Lee, R., Papa,R., McClean, P. Investigation of the domestication of common bean (*Phaseolus vulgaris*) using multilocus sequence data. Functional Plant Biology (published on-line November 7, 2011)

Oral/ Poster Presentations:

Gepts, P., Lin, D. Boveda, J., Britton, M., Fass, J., Navarro Gomez, A., Joshi, N., Kami, J., Lei, S., Lu, Z.-W., Repinski, S. 2011. Phaseolusgenes, a genome database for Phaseolus vulgaris: SSR marker discovery based on methyl-filtrated BAT93 genomic DNA and incorporation of comprehensive QTL information. Plant and Animal Genome Conference Abstract, January 2011.

Moghaddam, S.M., Mamidi, S., Lee,R., Osorno, J. McClean, P.E. 2011Plant and Animal Genome Conference Abstract, January 2011. Dual purpose markers for genetic and genome analysis.

McClean, P.E. 2011. Applications of the common bean genome. Bean Improvement Cooperative. November, 2011.

Felicetti E., Song, Q., Jia, G., Cregan, P., Bett, K., Miklas, P. 2011 SSR markers linked with slow dark trait in pinto bean were discovered by SNP assay and whole genome sequence. Bean Improvement Cooperative. November, 2011.

Brick, L.A., M.A. Brick, D. Echeverria, H.J. Thompson and A.Kleintop. 2011. Variation for dietary fiber content in dry edible beans. Oral Presentation at the Western Society of Crop Science Meetings, June 20-22, 2011, Laramie, WY. *Presenter. Published abstract in American Society of Agronomy Abstracts, 2011, Madison, WI.

Brick, L.A., D. Echeverria, M.A. Brick. And H.J. Thompson 2011. Dietary fiber content of dry edible beans. Colorado Bean News Vol. 24 (2):6-7.

Brick, L.A., M.A. Brick, D. Echeverria, H.J. Thompson and A.Kleintop. 2011. Variation for dietary fiber content in dry edible beans. Oral Presentation at the biennial meeting of the Bean Improvement Cooperative. October 3- November 2, San Juan, Puerto Rico.

Carpenter, G., M.A. Brick, B. Ogg, and K. Cichy. 2011. Comparison of Seed Coat Luster in a Recombinant Inbred Line of Shiny and Opaque Black beans. Agronomy Abstracts. Presented at the annual meeting of Crop Science Society of America, October 16-20, 2011 San Antonio, Texas.

Hueftle, S., P.R. Byrne, and M.A. Brick. 2011. Molecular Markers Linked to Quantitative Trait Loci For Resistance to *Fusarium* Wilt in Common Bean. Agronomy Abstracts. Presented at the annual meeting of Crop Science Society of America, October 16-20, 2011 San Antonio, Texas.

Community Resources Generated:

BeanCAP SNP Set: In collaboration with the Generation Challenge Program, the BeanCAP project released 1536 SNPs to KBioscience for the development of the common bean KSpar marker set.

Training:

North Dakota State University: Bradley Bisek, Undergraduate intern; Nicole Dallman, Undergraduate intern; Kataryna Cookman, Undergraduate intern; Mitchell Bauske, Undergraduate intern; Lyndsie Park, high school intern; Peter Totten, high school intern; Christina Johnson, Artistic lead; Shane Reetz, Documentary lead; Bree Malingnen, Infrographics artist; Samira Mafi Moghaddam, Marker development; Rian Lee, Marker development; Sujan Mamidi, Statistical analysis; Stacy Halvorson, extension associate; Leah, Whigham, USDA/Grand Forks, nutrition researcher; Deb Habedank, childcare director; Todd Weinmann, extension agent; Steve Sagaser, extension agent; Chelsea Langus, undergraduate student intern; Alexandra Idso, undergraduate student intern; Aimee Henning, undergraduate student intern, Kendra Otto, undergraduate student intern; Emily Westrom, undergraduate student intern; Amy Hutchinson, undergraduate student intern; Kayla Bahtiraj, undergraduate student intern; Co-PD; **University Nebraska, Lincoln:** Nicole Schnitger, Undergraduate summer intern student; Misty Griffitts, Undergraduate summer intern student; Scout Wilson, High school intern; Charity Berkey, Undergraduate summer intern student; Danielle Becker, Undergraduate summer intern student; **Baylor College of Medicine:** Paz Etcheverry, cooperator; **USDA/Houston:** David

Dworak, research technician; Lori Center, research technician; William Carter, summer intern; **Oregon State University:** Annie Chozinski, faculty research assistant; Kara Young, undergraduate intern; Katrina Maguelli, undergraduate intern; **University of California, Davis:** Shelby Repinski, graduate student, QTL entry to database; Adriana Navarro Gomez, graduate student, QTL entry to database; Sun Lei, graduate student; Tania Gioia, graduate Student; Dawei Lin, Bioinformatics and database lead; Jose Boveda, database/web programmer; Joe Fass, lead programmer; Nikhil Joshi, bioinformatics programmer; Monica Britton, bioinformatics analyst; Zhi-Wei Lu, bioinformatics analyst; **Michigan State University:** Evan Wright, research technician; Amy Lasley, graduate student; Valerio Hoyos Villegas graduate student; Rosa Castanon, high school intern; Brittany Lane, high school intern; Susan Swanson, USDA/Prosser, research technician; **USDA/Prosser:** Jennifer Trapp, research technician; Jeff Coulson, research technician; **USDA/Beltsville:** Edward Fickus, marker development technician; Qijiain Song, bioinformatics analysis; Gaofeng Jia, marker and bioinformatics analysis; Charles Quigley, research DNA sequencing; **Federal University of Vicosa, Brazil:** Josaine Rodriques, SSR analysis; **Colorado State University:** Soni Hueftle, undergraduate plant breeding intern; Griffin Carpenter, undergraduate plant breeding intern; Keera Brown, high school plant breeding intern; Alyssa Bollig, high school plant breeding intern; Dimas Echeveria Moreno, research associate, nutrition analysis. **USDA/Mayaquez:** Abraham Montes, research technician; Franquie Colon, research assistant; Gregory Howard, research; Edlin Gonzalez, research.

Collaborations:

1. Generation Challenge Program

2. US/AID Pulse CRSP Project

Development of a High Resolution SNP Chip (600K) and Genotyping of 850 Diverse rice (Oryza sp.) Accessions

Susan McCouch

Project Director: Susan McCouch, Cornell University, srm4@cornell.edu
Co-PD: Edyth Paul, GeneFlow, Inc., ediepaul@earthlink.net

Project website: www.ricesnp.org

Objectives and Accomplishments:

The project consists of three major activities: 1) design and manufacture a 600K SNP chip for rice; 2) prepare, genotype and analyze 850 diverse rice DNA samples; 3) develop training/educational materials.

Activity 1) The 600K SNP rice genotyping array was designed during 2010 from a pool of SNPs developed by the International Rice SNP Consortium. **Activity 2:** High quality DNA samples were extracted from ~450 diverse *O. sativa* and ~100 *O. rufipogon* accessions and used for genotyping with the 600K chip. A new version of our in-house allele-calling software ALCHEMY was developed to call the SNPs on the array. The 550 lines had been previously phenotyped as the basis for genome wide association analysis. To evaluate the methylation status of rice seedlings, we grew 11 diverse rice varieties under stress (heat, cold, Al, Cd, As) and control conditions, and DNA samples from individual plants were digested with either *HpaII* (methylation-sensitive), *Msp1* (methylation-sensitive), or *HaeIII* (methylation-insensitive), to enable us to identify variation in methylation status throughout the rice genome in response to stress. **Activity 3:** We participated in a 1 1/2-day workshop in Stuttgart, AR on February 8-9, 2011 that was run in collaboration with the USDA-ARS rice community, and in a 3 day mini-course on March 8-10, 2011 that was held at IRRI, Philippines. The software component of the training module was developed by Edyth Paul.

Broad Impacts:

The over-arching goal of this proposal is to develop a SNP chip and data resource that will empower the rice community to readily integrate modern molecular breeding technologies with classical breeding practice. By providing a diversity dataset on a large collection of rice germplasm, we aim to enhance the rice community's efforts to immediately apply marker-assisted selection in breeding, as well as to undertake QTL and association mapping, mutant analys, gene discovery, germplasm characterization, comparative and evolutionary studies. The unanticipated outcomes of the project will likely derive from empowering basic research discoveries that link sequence and epigenetic variation with phenotypic diversity associated with physiological response, plant development and agronomic traits, increasing our understanding of the biological role of genomic sequence.

Deliverables:

Publications: We aim to have a major publication in 2012 (under our no-cost extension). Our collaboration with IRRI (to collectively genotype 2,000 rice samples) greatly expanded the dataset but it also caused a delay in our expected time-frame which has significantly expanded the scope and impact of the project.

Oral/ Poster Presentations:

McCouch et al. (2010) "Development of a high resolution SNP chip (600K) and genotyping of 850 diverse rice (*Oryza sp.*) accessions", Poster presented at PAG 2010.

McCouch et al., (2011) "Development of a high resolution SNP chip (1M) and genotyping of diverse rice (*Oryza sp.*) accessions". Poster presented at PAG 2011.

Wright, Tung and McCouch (2011) "PANATI: Highly sensitive and computationally efficient analysis of next-generation sequencing data in *Oryza sativa* for SNP discovery, RNA-seq, CNV detection, genotyping by sequencing, and population genetic analysis", Poster at PAG, Jan. 2011.

Community Resources Generated:

The two software pipelines developed with support from this grant, ALCHEMY and PANATI, are available for use, free to both academic and commercial users under the GNU public license, at http://alchemy.sourceforge.net/ and http://panati.sourceforge.net/. A peer-reviewed publication on PANATI is in preparation at this time.

The raw sequencing data and the derived ~24M SNPs produced by the PANATI analysis of the data are currently available to Rice SNP Consortium members through the Amazon EC2 "Cloud". Due to the large volume of raw data and the significant computational resources needed to analyze it, it is anticipated that releasing the data in the Amazon cloud represents a convenient option for users, as downloading the entire dataset would not be necessary to analyze or use the data (i.e., it can be directly accessed from Amazon EC2 nodes).

Purified seed stocks from the ~450 *O. sativa* lines being genotyped on this project are available from the Genetic Stocks *Oryza* Center at the Dale Bumpers National Rice Research Center in Stuttgart, AR and along with the 1,440 purified lines developed at IRRI, through the International Rice Genetic Resources Center (IRRI) in the Philippines.

Training:

Mark Wright, former PhD Student, Dept Computational Biology and Biological Statistics, Cornell University; currently a Research Associate in the Dept. of Plant Breeding and Genetics on this project. Mark is responsible for a) developing the suite of algorithms (PANATI) used to generate the SNP discovery pool; b) inventing and implementing the strategy used to design the 1M SNP chip; and c) developing and implementing the algorithm used to call SNPs coming off the 1M array (ALCHEMY).

Chih Wei Tung, Research Associate in the Dept Plant Breeding & Genetics, is responsible for a) project management, b) developing protocols for genotyping of rice samples using Affymetrix fixed arrays, b) growing plants, harvesting tissue and making of all the paired end libraries used for re-sequencing to generate the SNP discovery pool, c) extracting 650 DNA samples for genotyping using the 1M SNP chip on this project, d) tracking of all DNA samples from off-site collaborators throughout the project,.

Adrian Powell, PhD student in the Dept. Plant Biology, Cornell University, trained as a rotation student in the McCouch lab using the re-sequencing data to analyze the origin of domestication alleles and to develop smaller SNP assays for tracking introgressions.

Maria Carrizales, PhD student in the Dept. Plant Biology, Cornell Univ, is managing plant stress experiments to evaluate epigenetic variation in plant response to environmental stress.

Kazi Akther, Research Technician, Dept. Plant Breeding & Genetics, Cornell University, assists with growing of plants in the greenhouse, DNA extractions, data management.

Collaborations:

1) **Global Rice Science Partnership** (http://irri.org/our-science/global-rice-science-partnership-grisp) is a large research initiative in rice that brings together three international centers, IRRI, CIAT and AfricaRice. Outputs from this USDA project are counted as deliverables under Theme 1 of the GRiSP. As a result of the close working relationship, McCouch was invited to serve on the Scientific Oversight Committee for the GRiSP. The McCouch lab was also invited to join a small exploratory project (funded by the US-AID Linkage program with IRRI) to help develop a database for the rice community at large that will provide public access to the genotyping and phenotyping data being generated on this and other projects.

2) **Expression Analysis** (http://www.expressionanalysis.com/) in North Carolina, US (CEO: Steve McPhail), and **DNA Landmarks** (http://www.dnalandmarks.ca/english/) in Montreal, Canada (CEO: Charles Pick) are commercial genotyping service providers who worked with us to standardize the DNA prep protocols and prices charged to the public. By working closely with these groups, we do our best to guarantee high quality genotyping and rapid turn-around-time on rice samples being genotyped using our arrays.

3) The **Rice SNP Consortium** (www.ricesnp.org) represents 14 different research groups from 10 different countries that worked together and contributed funds or raw re-sequencing data (fastq files) to establish the pool of SNPs required to build the 600K SNP chip. This consortium is enthusiastic about the project and is interested in using the 600K SNP chip as soon as possible.

4) **Affymetrix** (Julie Montgomery, Anne Ferguson, Michael Christiaens) helped us optimize the DNA prep protocols where enzyme digestion was not required prior to hybridizing onto the arrays. This saved significantly in the cost of the DNA prep and Affymetrix developed new reagent kits for rice that are different from those required for human and large genome species.

We are also working with Affy's International Marketing group (Rob Henke) to identify high quality genotyping providers in China, India, Thailand and Japan so that rice researchers in these countries can genotype rice samples using our chips without having to ship rice DNA or seeds out of their countries (which is normally not allowed). This helps to create new markets for the chips we have designed and helps to highlight the quality of both the chips and the datasets we have created on this project.

Barley Coordinated Agricultural Project

Gary J. Muehlbauer

Project Director: Gary J. Muehlbauer, University of Minnesota, muehl003@umn.edu

Co-PDs: Byung-Kee Baik, Washington State U.; Rex Bernardo, U. of Minnesota; Tom Blake, Montana State U.; Peter Bradbury, USDA-ARS, Ithaca, NY; Victoria Blake, Montana State U.; Shiaoman Chao, USDA-ARS, Fargo, ND; Timothy Close, UC, Riverside; Blake Cooper, BARI; Julie Dickerson, Iowa State U.; Ruth Dill-Macky, U. of Minnesota; Carl Griffey, Virginia Tech; Patrick Hayes, Oregon State U.; David Hole, Utah State U.; Richard Horsley, North Dakota State U.; Lee Jackson, UC, Davis; Jean-Luc Jannink, USDA-

ARS, Ithaca, NY; Jennifer Kling, Oregon State U.; Peggy Lemaux, UC, Berkeley; Stefano Lonardi, UC, Riverside; David Matthews, USDA-ARS, Ithaca, NY; Stephen Neate, North Dakota State U.; Donald Obert, USDA-ARS, Aberdeen, ID; Mark Schmitt, USDA-ARS, Madison, WI; Paul Schwarz, North Dakota State U.; Kevin Smith, U. of Minnesota; Brian Steffenson, U. of Minnesota; Steven Ullrich, Washington State U.; Mitch Wise, USDA-ARS, Madison, WI; Roger Wise, USDA-ARS, Ames, IA

Project website: www.barleycap.org

Objectives and Accomplishments:

Objective 1. High-Throughput Marker Development.

SNP platform development and mapping. The original objective was to develop a SNP map and genotyping platform for barley and integrate the SNP with the BAC-based physical map. As described in previous progress reports this objective was completely met. An international SNP platform composed of 3,072 SNPs was developed and 2,943 were mapped in four populations (Close et al., 2009). Various groups have utilized this map for numerous genetics and genomics projects. The maps were distributed and placed on THT (now T3, see below), HarvEST:Barley (http://harvest.ucr.edu/) and GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml). An improved consensus map was completed based on re-analyzing all data used previously and adding data from eight additional populations (Muñoz-Amatriaín et al., 2011). A total of 1,960 BOPA1 and BOPA2 SNPs have been anchored directly to 2,522 gene-bearing Morex BACs. Integrating these 2,522 BACs with 83,831 fingerprinted BACs anchored approximately 20,000 gene-bearing Morex BACs to the genetic map.

Genotyping-by-sequencing. We developed a novel two-enzyme genotyping-by-sequencing (GBS) protocol and genotyped bi-parental barley reference populations to develop a genetically anchored reference map of identified SNPs and tags. We were able to map over 56,000 SNPs and 289,000 tags (as dominant markers) onto the Oregon Wolfe Barley reference map and the Morex x Barke reference map, respectively. We have developed an initial map of the GBS tags in the barley genome and this genetic map is being used to anchor the barley integrated physical map. Further, the GBS approach developed here for barley is able to provide tens of thousands of markers at a per sample cost below $20. This GBS method therefore has great utility in barley genetics studies and barley breeding for genomic selection.

Objective 2. Worldwide Web Access.

The Hordeum Toolbox (THT) database. The original objective was to develop a database to house and display data from the barley CAP and to provide downloads. Data from the barley CAP are available in THT. THT has been adopted by the Triticeae CAP (TCAP) and has been renamed The Triticeae Toolbox (T3; http://triticeaetoolbox.org/) with a specific portion of the database called T3-barley. T3-barley houses the barley CAP data and in the future will house all TCAP data. The oat community has also adopted the THT schema and has established a database called The Avena Toolbox (http://avena.pw.usda.gov/tat/). The adoption of THT by the TCAP and the oat community indicates that the development and utility of the database has been a success.

Objective 3: Genes and Traits.

The original objective was to phenotype and genotype 96 advanced breeding lines each year from each of the ten breeding programs and use association mapping strategies on the combined datasets to identify marker-trait associations.

Germplasm. Each breeding program submitted 96 advanced breeding lines each of the four years (960 lines/year) for a total of 3,840 lines. Phenotypic data was obtained from each of these lines for approximately 30 traits including: morphological traits, agronomic traits, biotic and abiotic stress, grain quality traits, food quality traits, and malting quality traits. SNP genotyping with 3,072 SNPs was completed on each of the lines.

Association Mapping. QTL associated with Fusarium head blight resistance, resistance to deoxynivalenol accumulation, Ug99 resistance, common root rot resistance, net-form of net blotch resistance, spot blotch resistance, preharvest sprouting, height, heading date, yield, malting quality traits, food quality traits and winter hardiness have been identified. Multiple papers have been published, are in press or in preparation that highlight this work.

Genomic selection and association mapping theory. Multiple papers have been published that used barley CAP data to examine approaches to conduct genomic selection and the parameters for association mapping.

Objective 4: Superior Germplasm. This objective was focused on developing varieties via marker-assisted selection strategies.

Hayes and Cuesta-Marcos (Oregon State U.). MAS was used to develop winter habit waxy food barley from winter (non-food, non-waxy) x spring (food, waxy) crosses. This effort resulted in two potential varieties. This work is one of two chapters in Yada Chutimanitsakun Ph.D. thesis. She will defend Fall quarter, 2011. A manuscript describing the project will be submitted in late 2011/early 2012. MAS was used to introgress three stripe rust resistant QTL into two susceptible varieties (Baronesse and Kurtford). The Baronesse project is described in Verhoeven et al., 2011. The Kurtford project was completed but the industry partner assumed control of the germplasm and has not shared results. A custom designed 384 custom SNP OPA was used for winter malting barley MAS. Selected lines were planted in a field trial in Fall, 2011. In collaboration with Kevin Smith and Jean-Luc Jannick we are participating in a genomic selection project for six-row malting barley with low temperature tolerance. In cooperation with Deven See, a range of malt and food barley lines have been genotyped at the USDA-ARS Pullman lab using Sequenom assays. Targets are low temperature tolerance, quality traits, and stripe rust resistance.

Hanning (Busch Agricultural Breeding Program). SNP markers have been converted to a KASP- labeled marker for end-point genotyping. Markers associated with traits of interest (net blotch, scald, pre harvest sprouting, DON concentration and malting quality) have been identified and genetic strategies to validate and utilize them are being developed. Highly polymorphic markers are being used in variety identification and breeder seed development projects.

Griffey (Virginia Tech). A marker-assisted backcross breeding method is being used to transfer the hulless trait into the high yielding hulled barley cultivar Thoroughbred. In the spring of 2010 BC_1F_1 plants derived from crosses between Thoroughbred and elite hulless lines (Dan, VA04H-53 and VA01H-125) were backcrossed to Thoroughbred. In addition, F_1 progeny derived from crosses between Thoroughbred and elite hulless line VA05H-147 (powdery mildew and leaf rust resistance) were backcrossed to Thoroughbred. Markers linked to the *nud* locus were used to identify individual backcross progeny having the hulless gene after each cycle of backcrossing. Disease (powdery mildew and leaf rust) screening of Thoroughbred type individuals, possessing the hulless trait allowed for removal of susceptible plants, therefore, eliminating several initial crosses. This past spring (2011), a second cycle of backcrossing began with BC_1F_1 plants derived from Thoroughbred x VA05H-147 and BC_2F_1 plants derived from crosses between Thoroughbred and elite hulless lines, DAN and VA01H-125. Additionally, F_1 plants derived from crosses between Thoroughbred and Eve (powdery mildew and FHB resistance) were backcrossed to Thoroughbred. This fall (2011) F_2, BC_1F_2, and BC_2F_2 seed collected from selfed backcross progeny were planted in head rows. These plants will be evaluated and desired types will be selected and advanced. Additionally, another round of marker-assisted selection and backcrossing has been initiated this fall. Backcrosses between BC_1F_1, BC_2F_1, and BC_3F_1 and Thoroughbred will be made this coming spring (2011). This will further improve yields obtained in Thoroughbred type barley lines under disease epidemics, and lead to the development of the next generation of high-quality disease resistance winter barley cultivars.

Blake (Montana State U.). MAS was successfully used to introgress the low grain protein content allele at *qGPC6H* into durable, highest yielding feed and malt varieties. MAS is also being used to develop barley straw into a viable bioethanol feedstock (e.g., high fructan content). Six marker loci that appear to contribute to high straw fructan content are being used to develop hay and feed barley varieties that contain these introgressed loci.

Ullrich (Washington State U.). MAS is being used to select for yield and malting quality QTLs from Baronesse / Harrington backcrosses. MAS is also being used to select for stripe rust resistance QTLs.

Horsley (North Dakota State U.). MAS is being used to select for reduced deoxynivalenol accumulation and net blotch and spot blotch resistance. In the near future, a MAS approach will be implemented for wort beta-glucan, and resistance to septoria and the spot form of net blotch.

Smith (U. of Minnesota). Genomic selection using a 384 SNP chip is being used to improve FHB resistance. One breeding cycle of selection is being conducted each year. Breeding lines from cycle 1 were grown in trials in the summer of 2011 and that data is being used to assess selection accuracy and gain from selection. Selection for cycle 2 is in progress. A 48 SNP chip was used to develop near isogenic lines to validate QTL that were identified by association mapping. Data analysis is still in progress, but several QTL have been validated with data analyzed to date.

Varieties released:

Kevin Smith - Six row spring malting varieties (Quest and Rasmusson)

Patrick Hayes	- Six row winter malting variety (Maja)
	- Six row winter forage (Verdant)
Tom Blake	- Two row dryland malting variety (Hockett)
	- Hooded hay variety (Lavina)
Richard Horsley	-Potential six row spring malting releases (ND20493 and ND20448)
Carl Griffey	- Hulless variety (Eve)
	-Hulled variety (Atlantic)
Don Obert	- Two row spring feed (Tetonia)
	- Two row winter malt (Endeavor)
	- Two row spring feed (Lenetah)
	- Two row spring (Transit)
	- Two row spring (Julie, in process of being released)
Blake Cooper/Gary Hanning	- Two row spring malting (Merit 57 and Merit 16)
	- Six row spring malting (Celebration and Innovation)

Objective 5: Education and Outreach. Numerous activities have been conducted to extend information to graduate students, postdocs and breeders and to growers. Many of these activities were described in previous progress reports.

Materials. A podcast on Ug99 stem rust was completed and is available on YouTube (http://www.youtube.com/watch?v=o6ZkJCXydn8) and University of Minnesota website (http://plpa.cfans.umn.edu/). The Barley CAP coordinated its extension and education efforts with the Institute of Barley Malt Sciences (IBMS, http://www.ag.ndsu.edu/ibms/) at North Dakota State University. Thus far in 2011, one IBMS newsletter and thirteen press releases were developed that targeted growers and industry representatives.

Meetings. The IBMS planned and hosted two meetings called "Best of the Best Research in Wheat and Barley" in Minot and Bismarck, ND and were attended by more than 170 producers. The IBMS also assisted with the Sugarbeet Symposium in Billings, MT and the Small Grains Symposium in Great Falls, MT. The IBMS, in collaboration with the American Malting Barley Association (AMBA), has also developed extension teams composed of growers, end users and extension educators in Montana, North Dakota and Idaho.

eXtension. We worked with SolCAP to prepare the Barley CAP eXtension web page for barley growers (http://www.extension.org/pages/32458/barley-information-for-growers) in preparation for its official launch in January 2011 in San Diego, CA. A talk on the barley page was presented at the formal launch event. As part of the launch, a press release was prepared by the IBMS. A set of FAQs regarding barley was posted to eXtension. An article describing the launch was published in the Idaho Barley Newsletter. There is a news feature associated with the Plant Breeding and Genomics homepage on eXtension that is available to post news stories that might be of interest to users of the website; however, it was discovered that barley news articles that were posted were getting lost among the other non-barley related material. Thus, a page dedicated to news articles that would be of interest specifically to barley growers was created. We also created a blog page, which is linked to the growers' eXtension page, in order to keep all news articles of interest to the growers easily accessible to them. We also checked user stats for the barley pages at the request of SolCAP to evaluate high-performing content and sent that data to SolCAP. The barley grower page was linked to the barley Wikipedia page (http://en.wikipedia.org/wiki/Barley). With the ending of BarleyCAP efforts on eXtension, responsibility for its upkeep was transferred to the IBMS (Hertsgaard). Hertsgaard continues to serve on the PBGworks Content Committee to maintain the infrastructure for the barley grower pages and updating any links.

Broad Impacts:

1. Barley OPA1 and OPA2 are serving as an international platform for SNP genotyping.

2. Two association mapping workshop were conducted and attended by students, postdocs and breeders.

3. An analysis workshop to analyze barley CAP data was hosted.

4. Materials for the COP on eXtension were developed and implemented.

5. The IBMS and extension teams have implemented extension plans and hosted producer meetings.

6. THT was adopted by the oat and wheat communities.

7. PIs of the barley CAP obtained additional USDA-NIFA funding for four grants including a barley sequencing project, a genomic selection project, a winter barley project, and the Triticeae CAP, and approval for a DOE JGI barley sequencing project.

Deliverables:

Developed BOPA1 and BOPA2 (3,072 SNPs of which 2,994 are mapped).

Genotype data for 3,072 SNP alleles from 3,840 breeding lines were obtained.

Integration of the SNP genetic map and BAC-based physical map.

56,000 and 289,000 GBS markers mapped on OWB and Morex x Barke maps, respectively.

5. THT is fully implemented at GrainGenes and has been adopted by the TCAP and the oat community.

6. Phenotype data for over 30 traits was collected for the 3,840 breeding lines.

7. Source seed for the 3,840 breeding lines has been stored at the USDA-ARS National Small Grains Collection at Aberdeen, ID.

8. QTL for Fusarium head blight resistance, DON resistance, spot blotch resistance, net blotch resistance, height, heading date, yield, Ug99 resistance, common root rot resistance, malting quality traits, preharvest sprouting, and winter hardiness have been detected.

9. Marker-assisted selection is being conducted in eight breeding programs.

10. Genomic selection strategies have been implemented at the U. of Minnesota and Oregon State U.

11. Developed and distributed extension materials.

12. Extension teams in Montana, Idaho, and North Dakota have implemented extension plans.

13. Hosted two association mapping workshops and a one-day analysis session for the project.

Publications (32 papers have been published in previous years):

Brooks, W. S. M. E. Vaughn, C. A. Griffey, W. E. Thomason, J. J. Paling, R. M. Pitman, D. W. Dunaway, R. A. Corbin, J. C. Kenner, E. G. Hokanson, H. D. Behl, B. R. Beahm, S. Y. Liu, P. G. Gundrum, A. M. Price, D. E. Brann, D. L. Whitt, J. T. Custis, D. E. Starner, S. A. Gulick, S. R. Ashburn, E. H. Jones Jr., D. S. Marshall, M. O. Fountain, T. D. Tuong, D. P. Livingston, R. Premakumar, M. J. Kurantz, F. Taylor, R. A. Moreau, and K. B. Hicks. 2011. Registration of 'Dan' Winter Hulless Barley. J. Plant Reg. 5: 1-4.

O'Boyle, P. D., W. S. Brooks, B. J. Steffenson, E.L Stromberg, and C. A. Griffey. 2011. Genetic characterization of barley net blotch resistance genes. Plant Disease 95:19-23.

Verhoeven, E. C., M. Bonman, P. Bregitzer, B. Brunick, B. Cooper, A.E. Corey, A. Cuesta –Marcos, T. Filichkina, C.C. Mundt, D. Obert, B. Rossnagel, K. Richardson, and P.M. Hayes. 2011. Registration of the BISON genetic stocks in *Hordeum vulgare* L. J. Plant Reg. 5:135-140.

Lorenz, A.J., M.T. Hamblin, and J.-L. Jannink. 2010. Performance of single nucleotide polymorphisms versus haplotypes for genome-wide association analysis in barley. PLoS ONE 5(11): e14079.

Muñoz-Amatriaín, M., Y. Xiong, H. Bilgic, A.D. Budde, M.R. Schmitt, K.P. Smith, and G.J. Muehlbauer. 2010. Transcriptome analysis of a breeding program pedigree examines gene expression diversity and reveals target genes for the improvement of malting quality. BMC Genomics 11:653.

Comadran, J., L. Ramsay, K. MacKenzie, P. Hayes, T.J. Close, G. Muehlbauer, N. Stein and R. Waugh. Patterns of polymorphism and linkage disequilibrium in cultivated barley. Theor. Appl. Genet. 122:523-531.

Cistué, L., S. Chao, Y. Chutimanitsakun, A. Corey, A. Cuesta-Marcos, B. Echávarri, T. Filichkina, N. Garcia-Mariño, I. Romagosa, and P.M. Hayes. 2010. Comparative mapping of the Oregon Wolfe Barley using doubled haploid lines derived from female and male gametes. Theor. Appl Genet. 122:1399-1410.

Bradbury, P.J., T. Parker, M.T. Hamblin, and J.-L. Jannink. 2011. Assessment of power and false discovery in genome-wide association studies using the BarleyCAP germplasm. Crop Sci. 51:52-59.

Cuesta-Marcos, A., P. Szucs, T.J. Close, T. Filichkin, G.J. Muehlbauer, K.P. Smith and P.M. Hayes. 2011. Genome-wide SNPs and re-sequencing of growth habit and inflorescence genes in barley: implications for association mapping in germplasm arrays varying in size and structure. BMC Genomics 11:707.

Nair, S., Ullrich, S. E., and Baik, B.-K. 2011. Association of barley kernel hardness with physical grain traits and food processing parameters. Cereal Chem. 88:147–152.

Ramsay, L., J. Comadran, A. Druka, D.F. Marshall, W.T.B. Thomas, M. Macaulay, K. MacKenzie, C. Simpson, J. Fuller, A. Roberts, P.M. Hayes, U. Lunqvist, J.D. Franckowiak, T.J. Close, G.J. Muehlbauer and R. Waugh. Parallel selection at orthologous loci during the domestication of the grasses. Nature Genetics 43:169-172.

Sato, K., T.J. Close, P. Bhat, *M. Muñoz-Amatriaín* and G.J. Muehlbauer. 2011. Single nucleotide polymorphism mapping and alignment of recombinant chromosome substitution lines in barley. Plant and Cell Physiology 52:728-737.

Chutimanitsakun, Y., R.W. Nipper, A. Cuesta-Marcos, E.A. Johnson, and P.M. Hayes. 2011. Construction and application of a Restriction Site Associated DNA (RAD) linkage map in barley. BMC Genomics 12:4.

Lorenz, A. J., S. Chao, F.G. Asoro, E.L. Heffner, T. Hayashi, H. Iwata, K.P. Smith, M.E. Sorrells and J-L. Jannink. 2011. Genomic Selection in Plant Breeding: Knowledge and Prospects. Advances in Agronomy 110:77-123.

Hamblin, M.T., E.S. Buckler, and J.-L. Jannink. 2011. Population genetics of genomics-based crop improvement methods. Trends in Genetics 27:98-106.

Hamblin, M.T., and J.-L. Jannink. 2011. Factors affecting the power of haplotype markers in association studies. Plant Gen. 4:145-153.

Iwata, H., and J.-L. Jannink. 2011. Accuracy of genomic selection prediction in barley breeding programs: a simulation study based on the real single nucleotide polymorphism data of barley breeding lines. Crop Science 51:1915-1927.

Heffner, E.L., J.-L. Jannink, H. Iwata, E. Souza, and M.E. Sorrells. 2011. Genomic selection accuracy for grain quality traits in biparental wheat populations. Crop Science 51:2597-2606.

Heffner, E.L., J.-L. Jannink, and M.E. Sorrells. 2011. Genomic selection accuracy using multifamily prediction models in a wheat breeding program. Plant Gen. 4:65-75.

Russell, J., Dawson, I. K., Flavell, A. J., Steffenson, B., Weltzien, E., Booth, A., Ceccarelli, S., Grando, S., and Waugh, R. 2011. Analysis of >1000 single nucleotide polymorphisms in geographically matched samples of landrace and wild barley indicates secondary contact and chromosome-level differences in diversity around domestication genes. New Phytologist 191:564–578.

Newton. A. C., Flavell, A. J., George, T. S., Leat, P., Mullholland, B., Ramsay, L., Revoredo-Giha, C., Russell, J., Steffenson, B. J., Swanston, J. S., Thomas, W. T. B., Waugh, R., White, P. J. and Bingham, I. J. 2011. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. Food Sec. 3:141–178.

Elshire R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379.

Wu Y, Close TJ, Lonardi S. 2011. Accurate construction of consensus genetic maps via integer linear programming. IEEE/ACM Transactions on Computational Biology and Bioinformatics 8:381-394.

Nair, S., M. Knoblauch, S.E. Ullrich, and B.-K. Baik. 2011. Microstructure of hard and soft kernels of barley. J. Cereal Sci. doi: 10.1016/j.jcs.2011.06.014.

Wang, H., K.P. Smith, E. Combs, T. Blake, R. Horsley and G.J. Muehlbauer. 2011. Effect of population size and unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. Theor. Appl. Genet. DOI 10.1007/s00122-011-1691-8.

Gutiérrez L., A. Cuesta-Marcos, A. J. Castro, J. von Zitzewitz, P. M. Hayes. Association mapping of malting quality QTL in winter barley: positive signals from small germplasm arrays. The Plant Genome. In press.

Muñoz-Amatriaín M., M. J. Moscou, P. R. Bhat, J. T. Svensson, J. Bartoš, P. Suchánková, H. Šimková, T. R. Endo, R. D. Fenton, Y. Wu, S. Lonardi, A. M. Castillo, S. Chao, L. Cistué, A. Cuesta-Marcos, K. Forrest, M. J. Hayden, P. M. Hayes, R. D. Horsley, A. Kleinhofs, D. Moody, K. Sato, M. P. Vallés, B. B. H. Wulff, G. J. Muehlbauer, J. Doležel, T. J. Close. 2011 An improved consensus linkage map of barley based on flow-sorted chromosomes and SNP markers. The Plant Genome. 4:238-249.

Poland, J.A., P.J. Brown, M.E. Sorrells, J-L. Jannink. Development of high-density genetics maps for barley and wheat using genotyping-by-sequencing. Accepted PLoS One

Liang, M., D. Hole, J. Wu, T. Blake, and Y. Wu. 2011. Expression and functional analysis of NUCLEAR FACTOR-Y, subunit B genes in barley. Planta (Accepted)

Endelman, J.B., S. Nair, S. Chao, S.S. Jones, G.J. Muehlbauer, S.E. Ullrich and B.-K. Baik. Association mapping and genomic prediction of barley food quality in U.S. breeding lines. In preparation for submission to Crop Sci.

Blake, V.C., J.G. Kling, J-L. Jannink, G.J. Muehlbauer, K. Smith, P.J. Hayes, J. Lee, D.E. Matthews, R.P. Wise and J.A. Dickerson. The Hordeum Toolbox - The Barley CAP genotype and phenotype resource. In preparation for submission to The Plant Genome.

Book chapter

Thomas, W.T.B., P.M. Hayes, and L.S. Dahleen. Application of molecular genetics and transformation to barley improvement. 2011. In: Barley: Production, Improvement, and Uses. S.E. Ullrich (ed.) Wiley-Blackwell.

Non-refereed publications:

Ullrich, S. E., Baik, B.-K., Quinde-Axtell, Z., and Nair, S. 2010. Barley for food: Traits and improvement. p.563-575. In S. Ceccarelli and S. Grando, (ed.). Proc. 10th Int'l. Barley Genet. Symp. ICARDA, Aleppo, Syria. Available on line: http://icardablog.files.wordpress.com/2011/04/ibg10proceedings.pdf

Steffenson, B., G.J. Muehlbauer, K.P. Smith and P. Lemaux. 2010. Podcast on African Stem rust (http://www.youtube.com/watch?v=o6ZkJCXydn8)

Mamo, B., Brueggeman R., and Steffenson, B. 2011. Genetics of resistance to stem rust race TTKSK in barley landraces from Switzerland. Proceedings of 2011 BGRI Technical Workshop, St. Paul, Minnesota: 193.

Steffenson, B., Zhou, H., Chai, Y., Olivera, P., Capettini, F., and Grando, S. 2011. Vulnerability of *Hordeum* germplasm to wheat stem rust race TTKSK. Proceedings of 2011 BGRI Technical Workshop, St. Paul, Minnesota: 193.

One IBMS newsletters and thirteen IBMS press releases. http://www.ag.ndsu.edu/ibms/.

Presentations at scientific venues (88 presentations were given in previous years):

Gary Muehlbauer

Barley CAP Summary. 2011. Barley Improvement Conference, San Diego, CA

Improving barley and wheat germplasm for changing environments. 2011. Barley Improvement Conference, San Diego, CA

Genomics approaches to Triticeae improvement. 2011. Oregon State University, Corvalis, OR

Genome-wide association studies in barley: gene discovery and applications. 2011. University of Missouri, Columbia, MO

Genome-wide association studies in barley: gene discovery and applications. 2011. University of Georgia, Athens, GA

Patrick Hayes

Winter barley research update. 2011. Barley Improvement Conference, San Diego, CA

GMOs and Cereal Crops: Local to global issues. 2011. Sustainability in Agriculture Seminar series. Oregon State University, Corvallis, OR

Mitchell Wise

Barley Tocols: Potential for Food Barley Added Value (Tocols 101). 2011. North American Barley Researchers Workshop, Corvalis, OR

Jesse Poland

Genotyping-by-sequencing in barley and wheat. 2011. Barley Improvement Conference, San Diego, CA

Genotyping-by-sequencing for barley and wheat breeding and genetics. 2011. International Triticeae Mapping Initiative Summer Workshop, Mexico City, Mexico

Genotyping-by-sequencing for genomic selection in wheat and barley. 2011. University of Illinois, Champaign-Urbana, IL

Genotyping-by-sequencing for genomic selection in wheat and barley. 2011. Colorado State University, Fort Collins, CO

Kevin Smith

Keeping barley competitive through research: Genomics and breeding. 2011. Barley Improvement Conference, San Diego, CA

Jean-Luc Jannink

Population-specific research needs to launch genomic selection. 2010. National Marker-Assisted Selection for Crop Improvement, ICRISAT Patancheru, A.P., India.

Methods and breeding schemes applying genomic selection to crops in the public sector. 2011. Genomics Assisted Breeding Workshop, Plant and Animal Genome XIX, San Diego, CA.

Genomic selection for introgression and multi-parent populations for genomic selection. 2011. Multi-parent population workshop, CIMMYT, Mexico City, Mexico.

Applying Genomic Selection To Crops In The Public Sector. 2011. Genomic selection workshop, Meetings of the American Society of Agronomy, San Antonio, TX.

Steven Ullrich

Breeding barley to improve the diets of modern day Hordearii! (and everyone else). 2011. NABRW, Corvallis, OR.

Byung-Kee Baik

Food uses quality of barley. 2011. Barley CAP Annual Meeting, San Diego, CA.

Megan Lewis

Can mapping be done in a narrow cross? 2011. ASA abstracts, San Antonio, TX.

Richard Horsley

Status of research and development of malting barley cultivars in the USA (programs, linkages, and partnerships). 2011. Innovation Network Workshop sponsored by Impulsora Agricola S.A. (IASA) In Mexico City, Mexico

The collaborative FHB nursery at Zhejiang University and our outputs. 2011. Jiangsu Agricultural Academy of Sciences in Nanjing, China

Brian Steffenson

East African stem rust: research needs and challenges. 2011. 38th Barley Improvement Conference, San Diego, CA.

Association mapping of multiple disease resistance in US barley breeding germplasm. 2011. 20th North American Barley Researchers Workshop, Corvallis, Oregon.

Multiple disease resistance QTL analysis of two wild x cultivated barley populations. 2011. 4th International Workshop on Barley Leaf Blights. Dundee, Scotland

Barley Coordinated Agricultural Project: fermentation of ideas for effective outreach and education modules. 2011. Annual Meeting of the American Phytopathological Society, Honolulu, Hawaii.

Combating African stem rust: a global threat to food security. 2011. 1666 Coffman Group. St. Paul, MN

Genetic architecture of durable spot blotch resistance revealed by association mapping. 2011. Tel Aviv University, Tel Aviv, Israel.

Tim Close

Cowpea: genetic improvement for marginal environments. 2011. University of Nebraska, Lincoln, NE

Meeting Abstracts (54 abstracts were published in previous years):

Berger, G., S. Liu, M. Hall, W. Brooks, S. Chao, C. Griffey, and G. Muehlbauer. 2011. Identification of marker trait associations in the Virginia Tech winter barley breeding program using genome-wide mapping. American Society of Agronomy Meetings, San Antonio, TX

Zhou, H., B.J. Steffenson, G. Muehlbauer, R. Wanyera, P. Njau and S. Ndeda. 2011. Mapping and haplotype analysis of adult plant resistance to stem rust race TTKS in barley breeding germplasm from the USA. BGRI abstracts, St. Paul, MN

Wang, H., K.P. Smith, T. Blake, B. Cooper, D. Hole, R. Horsley, D. Obert, S. Ullrich, and G.J. Muehlbauer. 2011. Association mapping of grain yield and plant height in a drought trial in the barley coordinated agricultural project. Plant and Animal Genome Conference Abstracts. San Diego, CA.

Berger, G., S. Liu, M. Hall, W. Brooks, S. Chao, C. Griffey, G. Muehlbauer. 2011. Identification of marker-trait associations in the Virginia Tech barley breeding program using genome-wide mapping. In: Proceedings of the 2011 Eastern Wheat and Southern Small Grain Workers Conference; April 17-20; Grapevine, TX.

Poland, J., P. Brown, B. Steuernagel, N. Stein, M. Sorrells, J-L. Jannink. 2011. Genotyping-by-sequencing for barley and wheat breeding and genetics. International Triticeae Mapping Initiative Summer Workshop, Mexico City, Mexico

Poland, J., S. Dreisigacker, Y. Manes, J. Rutkoski, J. Dawson, J. Endelman, M. Sorrells, and J-L. Jannink. 2011. Genotyping-by-sequencing to enable genomics assisted breeding in wheat. Plant and Animal Genome Conference Abstracts, San Diego, CA

Vikram, V., R.D. Horsely, and K.P. Smith. 2011. Association-mapping for agronomic traits using elite breeding populations from two North American barley breeding programs. 2011. Plant & Animal Genomes Conference Abstracts, San Diego, CA

Correa-Morales, A.M., S. Chao, P. Werner, T. Blake, B. Cooper, D. Hole, D. Obert, K. Smith, S. Ulrich, and R.D. Horsley. 2011. Identification of QTL for Seed Dormancy in the Spring Barley CAP Lines. Pp 58. J. Nyachiro (ed.) Proc. 12 Intl. symposium on preharvest sprouting in cereals, Red Deer. Alberta.

Lewis, M.L., F. Pedraza-Garcia, R. Lin, S. Chao, P.B. Schwarz, and R.D. Horsley. 2011. Can mapping be done in a narrow cross? ASA abstracts, San Antonio, TX.

Correa-Morales, A.M., J. Jyoti, S. Chao, P. Werner, B. Cooper, K. Smith, and R.D. Horsley. 2011. QTL identification for seed dormancy in the Midwest spring barley CAP lines.

Lewis, M.L., F. Pedraza-Garcia, R. Lin, S. Chao, P.B. Schwarz, and R.D. Horsley. 2011. Mapping QTL controlling fermentable sugars in a narrow cross.

Community resources generated (sequences, populations, plant materials):

BarleyOPA1 and OPA2 (3,072 SNPs) are used as a standard genotyping platform.

Genetic map with 2,994 SNP markers with increased resolution.

1,960 BOPA1 and BOPA2 SNPs have been anchored to approximately 20,000 gene-bearing BACs.

56,000 and 289,000 GBS markers mapped on OWB and Morex x Barke maps, respectively.

Phenotype data for over 30 traits on 3,840 breeding lines.

Genotype data for 3072 SNP alleles on 3,840 breeding lines.

Genetically pure seed source for 3,840 breeding lines deposited at the USDA-ARS National Small Grains Collection at Aberdeen, ID

Extension/education materials (factsheets, brochure, podcast, posters, PowerPoint slides, newsletters)

Contributed to development of CoP for plant breeding and genomics for eXtension.

The THT database is online at http://www.hordeumtoolbox.org/, at GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and is the database for the TCAP (http://triticeaetoolbox.org/).

THT has been adopted by the oat community and called The Avena Toolbox (http://avena.pw.usda.gov/tat/).

Training:

Iowa State University

Shreyartha Mukherjee, Volodymyr Sukhoy, Kartic Ramesh, Suman Jillella, Yong Huang, Graduate Students with Dickerson.

Ethan Wilder, and Gavin Monroe, Undergraduate Students with Dickerson.

Shengqiang Zhong, Graduate Student with Jean-Luc Jannink.

Washington State University

Sindhu G. Nair, Graduate Student, with Baik and Ullrich.

Tracy Harris, Research Technologist, data management, with Baik and Ullrich.

Jennifer Anderson, Caitlin Rath, Elise Coons, Natalia Loukinova, Kyle Stokes, Brianna Tidball, Kasey Freston, Nicole Groth, Anna Gibson, Ryan Arnold, Undergraduate Students with Baik and Ullrich.

Wycliffe Nyongesa, Brenda Anderson, Research technician with Baik and Ullrich.

University of Minnesota

Lynne Medgaarden, Administrative Assistant with Muehlbauer

Sue White, Extension evaluator with Steffenson

Carol Powers, Jon Massman, Vikas Vikrum, Stephanie Navara, Graduate Students with Smith.

Ed Schiefelbein, Technician with Smith

Alyssa Bernardo, Magan Friskop, Undergraduate Students with Smith.

Hao Zhou, Ben Alsop, Graduate Students with Steffenson.

Joy Roy, Postdoctoral Research Associate with Steffenson.

Tamas Szinyei and Stephanie Dahl, Technicians with Steffenson

Srikanth Srinivasan, Srikrishnan Srinivasan, Vijay Ramadoss, Graduate Students with Bernardo.

Amar Elakked and Beheshteh Zargara, Technicians with Dill-Macky

Hongyun Wang and Maria Muñoz-Amatriain, Postdoctoral Research Associates with Muehlbauer.

Montana State University

Jeremy Jewell and Stan Bates, Chris Shafer and Duke Pauli, Graduate Students with Blake.

Victoria Carollo Blake, Research Assistant Professor

Jessica Patrick, Kile Patrick, Chris Shafer, Duke Pauli, Joe Lascerenza, Jen Holfeldt, Paige Tresitter, Andy Yates, Katarina Steele, Kelly Thornberry, Bradee Smith, Aiden Bickford, Nate Arthun, Jim Waterford, Undergraduates with Blake.

Oregon State University

Yada Chutimanitsakun, Juan Rey and Scott Fisk, Graduate Students with Hayes.

Alfonso Cuesta-Marcos and Peter Szucs, Postdoctoral Research Associates with Hayes.

Tanya Filichkina, Ann Corey and Kale Haggard, Technicians with Hayes

Dinara Andirova, Undergraduate student with Kling

Utah State University

Mingxiang Liang, Graduate student with Hole

Justin Clawson, Graduate Student now technician with Hole.

Corey Clawson, Undergraduate Student with Hole

Amelia Larned, High School Student with Hole.

North Dakota State University

Jennifer Bolivar and Paul Werner, Ana Correa-Moralea, Graduate Students with Horsley.

Jawahar Jyoti, Postdoctoral Research Associate with Horsley.

Mat Berghuis, Brent Horner and Kamal Thapa, Undergraduate Students with Neate.

Sanjaya Gyawali, Marcela Daza and Sharmila Sunwar Graduate Students with Neate.

Kyle Sebesta, Aaron Bedford, Michael Averson, and April Maertens, high school students with Neate.

Theja Wijetunga, Technician with Schwarz

Yin Li, Research Assistant Professor with Schwarz

Karen Hertgaard, Communication Specialist with Schwarz

USDA-ARS, Fargo, ND

Mary Osenga, Dawn Feltus, and Richard Sonju with Chao

Kristin Simons, Postdoctoral Research Associate with Chao

USDA-ARS, Ithaca, New York

Shengqiang Zhong, Graduate Student with Jannink.

Hiroyoshi Iwata, Visiting Professor, USDA-ARS, Ithaca, NY with Jannink.

Yi Jia, Postdoctoral Research Associate with Jannink.

Martha Hamblin, Senior Research Associate with Jannink and Bradbury.

Thomas Parker, Aaron Lorenz, Jesse Poland, Postdoctoral Research Associate with Jannink and Bradbury.

University of California-Riverside

Serdar Bozdag and Yonghui Wu, Graduate Students with Close and Lonardi.

Denise Duma, Graduate student with Lonardi

Prasanna Bhat, Livia Tommasini and Ndeye Diop, Postdoctoral Research Associates with Close.

Steve Wanamaker, Programmer with Close and Lonardi.

Raymond Fenton, Staff Research Associate with Close.

Yaqin Ma, Visiting Assistant Researcher with Close

Josh Resnik. Laboratory Assistant with Close.

Marti Pottorff, Graduate Student with Close.

Jessica Nguyen, Andrew Flores, Hana Abughoush, Undergraduate Students with Close

Matthew Alpert, Undergraduate student with Lonardi

Stephanie Chiueh, Junior Specialist with Close

James Roose, High School Student with Close

Joanna Werner-Fraczek, Community College Teacher with Close

University of California, Berkeley

Barbara Alonzo, Administrative assistant with Lemaux

Virginia Tech University

Patrick O'Boyle and Greg Berger, Graduate Students with Griffey.

Shuyu Liu, Research Scientist, with Griffey

Wynse Brooks, Research Associate with Griffey

Mark Vaughn and John Seago, Research Specialists with Griffey

USDA, Madison, Wisconsin

Hanna Tranel, Nick Cahill and Richard Keller, Undergraduate Students with Wise.

Collaborations. New interactions made possible from project support:

1. The development of the SNP genotyping platform and integration of SNPs with the physical map has created collaborations with the James Hutton Institute (Scotland), and the Institute of Plant Genetics and Crop Plant Research (Germany).

2. SNP map and integration with BAC-based physical map provided the opportunity to obtain additional funding from USDA-NFA for a project titled, "Advancing the Barley Genome" (PI: Close) and DOE-JGI-CSP approval to conduct a barley genome sequencing project.

3. Collaborated with SolCAP on developing community of practice for eXtension.

4. Collaborated with extension teams in Montana, Idaho and North Dakota.

5. The oat and wheat communities adopted THT for their database needs.

6. The THT schema has been adopted by the Triticeae CAP.

7. The barley CAP germplasm has been used to create association mapping panels that are being used in the Triticeae CAP.

8. The SNP platform was used to increase the resolution of the barley genetic map.

9. The breeders at Oregon State University and the University of Minnesota are collaborating to develop a winter barley breeding program at the University of Minnesota.

Conifer Translational Genomics Network Coordinated Agricultural Project

David Neale

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Project website: http://dendrome.ucdavis.edu/ctgn

Objectives and Accomplishments:

Objective 1: Association validation. Thousands of accessions from elite populations of pine and Douglas-fir were genotyped to verify associations discovered in experimental populations.

Objective 2: Development and economic evaluation of new methods incorporating marker-assisted selection into conifer tree breeding programs. A two-pronged approach was pursued: First, the points in time in the tree breeding cycle that MIB could be applied were defined and the manner in which they may be applied (i.e., forward or backward selection, informed breeding, informed project management applications, etc.) were assessed. Second, simulation software was developed with the Excel® add-in Simetar© to illustrate 1) possible implementations of MAS/MAB and 2) show how Monte Carlo simulation can be used to rank alternative breeding plans by expected economic returns.

Objective 3: Development of databases (TreeGenes) and web-based tools (Dendrome) to facilitate all aspects of the CTGN. A 'DNA Inventory/Sample Tracking Interface' was developed allowing all genotype data to be accumulated and stored in TreeGenes. A Plone content management system (Dendrome Plone) was implemented for CTGN project content. Genotype data delivered from Illumina is accumulated in the TreeGenes relational database, integrated with the available phenotypic data for these same individuals, and made available to project directors and the cooperatives for downstream

analyses. Extensive search interfaces were made available to allow bulk download as well as itemized searches. Data types available for search by project participants now include EST, EST contig, amplicon, sequence assembly, phenotype, sample identifiers, and SNPs. A search can begin at any of these levels and can yield detailed information on tracefiles, FASTA sequences, SNP scores, annotations, and genotypes.

Objective 4: International genetic stock center for conifers. There are three components: (1) the Loblolly Pine Genetic Stock Center, a clonal archive at the Southern Institute of Forest Genetics, Southern Research Station near Saucier, Mississippi, (2) the Douglas-Fir Genetic Stock Center, a clonal archive at the Pacific Northwest Research Station near Corvallis, Oregon, and (3) the UC Davis Forest Tree Genetic Stock Center, comprised of DNA stored in -80°C freezers (http://dendrome.ucdavis.edu/ftgsc/).

Objectives 5 and 6: Education and extension for genomics-based breeding in forest trees. Details available at http://dendrome.ucdavis.edu/ctgn/educationextension/

Training modules. As part of the Plant Breeding and Genomics Community of Practice with eXtension, a suite of 16 on-line modules covering genomics in tree breeding and ecosystem management, designed by CTGN to serve as complementary teaching aids for University instructors or as stand-alone lessons for students, practitioners, or curious laypeople were inaugurated in September 2011 (http://www.extension.org/pages/60370/).

Workshops. Three formal multi-day workshops were delivered to a total of some 75 students and practitioners and a number of one day workshops were delivered to cooperative members and symposia attendees. Details on all workshops, including access to teaching materials in some cases, are available at the project website.

University curriculum. Four new courses have been developed largely around materials and research of the CTGN, at three of our participating institutions, targeting both undergraduate and graduate level students. See website for details on the courses.

Graduate student training and internships. Six graduate students have received support from or worked on project data. Two interns were hosted for periods ranging from two to three weeks.

International symposium. CTGN investigators planned, hosted, and participated in the "Genomics-Based Breeding in Forest Trees", held June 22-24, 2012 at UC Davis, with 64 attendees. It was the first international meeting dedicated largely to applied genomic-based breeding in forest trees.

Outreach. The set of ten modules developed for the original CTGN workshop (UC Davis, June 15–19, 2009) is being dramatically revised into a 16-module set that will be available online, and possibly through eXtension, for long-term educational purposes. The modules will feature voice-over annotation of 30 selected slides per module.

Evaluation of Education and Extension activities. An independent evaluator, Dr. Michael Coe, Cedar Lake Research Group, Portland OR USA, carried out industry-wide surveys of tree improvement

programs, conducted interviews with tree improvement coop directors and staff members, and gathered feedback gathered from participants in CTGN-sponsored events and workshops. His reports are posted at the CTGN website.

Broad Impacts:

The SNP data generated by the project have been delivered to breeding coops leading to the beginning of a transition phase where these programs will now use genome-wide SNP data for Genomic Selection in addition to traditional phenotypic selection.

Deliverables:

Publications: The project website lists all published, submitted, and in progress publications.

Oral/Poster Presentations: Project investigators and graduate students have delivered nearly 100 presentations to all manner of audiences, from cooperative member meetings to classrooms to scientific meetings. A complete listing is available at the project website.

Community Resources Generated: The Infinium loblolly pine SNP genotyping chip generated by the project has now been used by private companies, for example, ArborGen.

Other products/outcomes: CTGN was recognized, September 14, 2011, with a 2011 US Department of Agriculture Secretary's Honor Award "*For a collaborative research and outreach approach to successful development and application of genomics-based tree breeding technology that will enhance US competitiveness in the production of forest products*." Five editions of a newsletter were produced and widely distributed as pdf attachments and from the project website.

Training: See above under Objectives 5 and 6.

Collaborations:

Two CTGN investigators (Neale and Wheeler) are participants in the FoResTTraC project (**Fo**rest ecosystem genomics **Res**earch: suppor**T**ing **Tra**nsatlantic **C**oopera

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Loblolly Pine Genome Project

David Neale

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Jill Wegrzyn, University of California, Davis, jlwegrzyn@ucdavis.edu
James Yorke, University of Maryland, College Park, yorke@ipst.umd.edu.edu

Project website (URL): http://pinegenome.org/pinerefseq/

Objectives and Accomplishments:

High-quality reference genome sequences of loblolly pine and two other conifer species, sugar pine and Douglas-fir. Relying on next-generation sequencing technology, novel cloning approaches, and new assembly algorithms, a sequencing approach for loblolly pine will be developed that can subsequently be used for any conifer species. As demonstration of the utility of this approach, it will be extended as part of this project to sugar pine and Douglas-fir. ***To date:***

1) New fosmid vectors have been developed and tested: one is being used specifically to create 40-kb paired-end loblolly pine libraries for Illumina sequencing, the others will be used for fosmid-pool loblolly pine genomic DNA sequencing.

2) Aspects of sequencing strategies have been investigated: rolling-circle amplification and its effect on sequence quality and sequencing of paired-end libraries from loblolly pine megagametophytes to assess library qualities and DNA isolation protocols.

3) Evaluation is underway of the available assembly packages for accuracy and ability to handle the large sequence data sets that conifer genomes present. Eight packages have been tested on Illumina data (Allpaths-LG, SOAPdenovo, ABySS, SGA, Velvet, MSR-CA, CABOG, and Bambus2). A new 'super-read' assembler (MSR-CA) is being developed and tested with the goal of using it on the loblolly pine sequence data. Assemblies have been carried out on the pine chloroplast genome, megagametophyte sequence data, and pine fosmid pool sequence data.

Transcriptome sequencing for gene discovery, reference building, and aids to genome assembly. To produce full transcript assemblies for functional genomics studies, RNAs will be isolated from a large number of loblolly pine organs, stages of development, and tissues exposed to biotic and abiotic stresses and then sequenced using the long reads of Roche/454 GS-FLX Titanium technology. ***To date:*** RNA libraries were prepared from seeds collected immediately after stratification, embryos dissected from seeds, and megagametophyte tissue and were sequenced and sequence was assembled. Analyses are underway to assess the extent of gene discovery possible in these libraries and to permit comparative analyses with other plant genome datasets. Other libraries under construction include immature pollen cones 5 to 7 mm long, vegetative buds from pollen cone shoots, tiny female buds (conelets) at various developmental stages, pollen strobili at various developmental stages, buds 5 to 10

mm long, vegetative buds in center of females, “candles”- shoot/green stem on which the female strobili were found, candles with vegetative and reproductive buds removed, female strobili at various developmental stages, terminal buds (center of female buds), and elongating lateral longer buds not associated with reproductive buds. Seed from the reference loblolly pine genotype has been obtained and is under stratification in preparation for library construction.

Dendrome and TreeGenes databases: Annotation, data integration, and distribution. The target is to develop easy-to-implement annotation pipelines and tools to fully integrate genome sequences into all other existing genomic information resources that can be used widely by, not only researchers, but also practical tree breeders and resource managers. ***To date:*** organization of existing transcriptome and genome resources and preparation of the TreeGenes/DiversiTree databases to accommodate the data anticipated from this project: the DNA inventory system has been updated to allow metadata information from RNA-Seq projects; integration of mapping data between DiversiTree and CMap has been tested, bulk download capacity has been reorganization with expansion of FTP resources, and discussions with the Plant Ontology project have been initiated to develop vocabularies for forest trees.

Training in genome sequencing, assembly, and analysis technologies and strategies. One two-day workshop consisting of lecture and practicum will be held in each of years 2 through 5 in this sequence: NGS technology applied to large genomes; genome assembly and mapping; annotation and transcriptome; and genome database and genome browser. ***To date:*** As planned, no activity in year 1.

Broad Impacts: Researchers in the conifer community will have access to pipelines, protocols, and genomic resources for work with large genomes. Because a strategic alliance has been formed with the horticultural genomics community through the Genome Database for Rosaceae (GDR) project, the strengths of both the forestry community and the horticultural community will be leveraged in this joint enterprise.

Deliverables:

Publications: None to date in year 1.

Oral/ Poster Presentations: A complete listing is available at the project website.

A ‘Pine Genome Reference Sequence’ Workshop has been organized for January 14, 2012 at PAG XX, during which presentations will be made on the above objectives and accomplishments as well as on progress in two parallel conifer genomics projects (Norway Spruce Genome Project and Canadian White Spruce Genome Sequencing Project).

Community Resources Generated: None to date in year 1

Other products/ outcomes: None to date in year 1

Training: None in year 1 per project timeline

Collaborations:

In addition to the planned collaboration with the USDA Douglas-fir climate change transcriptome observatory for the Pacific Northwest project (http://www.reeis.usda.gov/web/crisprojectpages/219761.html) and the Norway Spruce Genome Project (http://www.congenie.org/), additional collaborations have been established with the Canadian White Spruce Genome Sequencing Project (a component of SMarTForest: Spruce marker technologies for Sustainable Forestry; http://www.genomebc.ca/portfolio/projects/forestry-projects/smartforest/) and a project at the University of British Columbia (AdapTree: Assessing the adaptive portfolio of reforestation stocks for future climates; http://www.genomebc.ca/portfolio/projects/forestry-projects/adaptree/).

Scanning for Yield: High-Throughput Discovery of Candidate Agronomic Loci for Marker-Assisted Selection in Maize

Jeffrey Ross-Ibarra

Project Director: Jeffrey Ross-Ibarra

Project website: www.rilab.org

Objectives and Accomplishments:

Objective 1: Identify candidate agronomic loci.

We have genotyped a chronological sample of 400 North American corn belt lines for 46,000 single nucleotide polymorphisms. We have used these data to identify genomic regions that have changed in frequency due to likely selection. We have also detailed ancestry relationships along the maize genome. These data result in a list of candidate loci that appear to have been of agronomic importance (but are still polymorphic in modern inbred breeding lines), as well as the ability to assign a genotypic breeding value to individual lines based on their genotype.

Objective 2: Experimentally test candidate loci.

We have arranged a collaboration with Dr. Rita Mumm at the University of Illinois, which will allow us to test our candidate loci using an association analysis approach. Her data consist of hybrid yield trials of 12 inbred lines that we have genotyped. Additionally, we can make use of hybrid data from a large number of crosses performed using a maize association panel, for which we have genotype data for all lines.

Broad Impacts:

We have already identified a list of candidate genes that show evidence of selection during maize domestication and during subsequent improvement. Further work on these genes may validate many that could be of use to breeding.

We have developed novel statistical methods that can be applied to SNP data in other plant taxa for performing selection mapping or analysis of population structure.

We are expanding our work evaluating whether candidate loci show effects on yield to new projects aimed at understanding the genetic basis of heterosis (see collaborations below).

Deliverables:

Publications:

Hufford, MB, Xun X, van Heerwaarden J, Pyhäjärvi T, Chia J-M, Cartwright RA, Elshire RJ, Glaubitz JC, Guill KE, Kaeppler S, Lai J, Morrell PL, Shannon LM, Song C, Spinger NM, Swanson-Wagner RA, Tiffin P, Wang J, Zhang G, Doebley J, McMullen MD, Ware D, Buckler ES, Yang S, Ross-Ibarra J. Population genomics of domestication and improvement in maize. (Submitted)

Chia, J-M, Song C, Bradbury P, Costich D, de Leon N, Doebley JC, Elshire RJ, Gaut BS, Geller L, Glaubitz JC, Gore M, Guill KE, Holland J, Hufford MB, Lai J, Li M, Liu X, Lu Y, McCombie R, Nelson R, Poland J, Prasanna BM, Pyhäjärvi T, Rong T, Sekhon RS, Sun Q, Tenaillon M, Tian F, Wang J, Xu X, Zhang Z, Kaeppler S, Ross-Ibarra J, McMullen M, Buckler ES, Zhang G, Xu Y, Ware, D. Capturing extant variation from a genome in flux: maize HapMap II. (Submitted)

van Heerwaarden J, Hufford MB, Ross-Ibarra J. Historical genomics of North American maize. (Submitted)

Morrell PL, Buckler ES, Ross-Ibarra J. (2012) Crop genomics: advances and applications. Nature Reviews Genetics accepted

Cook, JP, McMullen JD, Holland JB, Tian F, Bradbury P, Ross-Ibarra J, Buckler ES, Flint-Garcia SA. Genetic architecture of maize kernel composition in the Nested Association Mapping and Inbred Association panels. In Press

van Heerwaarden J, Doebley J, Briggs WH, Glaubitz JC, Goodman MM, Sánchez González JJ, Ross- Ibarra J. (2011). Genetic signals of origin, spread and introgression in a large sample of maize landraces. PNAS 108: 1088-1092

Hufford MB, Gepts P, Ross-Ibarra J. (2011) Influence of cryptic population structure on observed mating patterns in the wild progenitor of maize (Zea mays ssp. parviglumis). Molecular Ecology 20: 46-55

van Heerwaarden J, Ross-Ibarra J, Doebley J, Glaubitz JC, Sánchez González J, Gaut BS, Eguiarte LE (2010) Fine scale genetic structure in the wild ancestor of maize (Zea mays ssp. parviglumis). Molecular Ecology 19: 1162-1173

Oral/ Poster Presentations (only 2011 shown):

Hufford, M.B., ..22 authors ... Ross-Ibarra, J. Genome-wide effects of domestication and improvement in landraces and modern maize. Oral presentation at the Maize Genetics Conference, Chicago, 2011.

Chia, J., ..10 authors.. Ross-Ibarra, J., McMullen, M.D., Buckler, E.S., and Ware, D. Maize HapMapV2 - Capturing variation in a genome in flux. Oral presentation at the Maize Genetics Conference, Chicago, 2011.

Pyhäjärvi, T., Hufford, M.B., and Ross-Ibarra, J. Genomic effects of local adaptation in Zea mays ssp. parviglumis populations. Poster presentation at the Maize Genetics Conference, Chicago, 2011.

van Heerwaarden, J, Hufford, M.B., and Ross-Ibarra, J. A genome-wide view of breeding history and selection in North American maize lines. Poster presentation at the Maize Genetics Conference, Chicago, 2011

Ross-Ibarra J. The domestication of maize: the where, the what, and the huh? Oral presentation at the ASA/CSSA/SSSA Convention, symposium on maize biology, San Antonio, 2011

Ross-Ibarra J. The population genomics of maize domestication and improvement. Oral presentation to the Dept. of Plant & Microbial Biology, UC Berkeley, 2011

Hufford, M, Lubinsky, P., Pyhäjärvi, T., Ellstrand, N, and Ross-Ibarra J. Dueling genomes: Reciprocal gene flow in hybrid swarms of maize and its wild relative, Zea mays ssp. mexicana. Oral presentation at the Society for the Study of Evolution conference, Oklahoma City, 2011

Pyhäjärvi, T., Hufford, M, and Ross-Ibarra J. Genomic effects of local adaptation in the wild relatives of maize. Oral presentation at the Society for the Study of Evolution conference, Oklahoma City, 2011

Community Resources Generated:

We have completed genotyping of ~400 lines of Zea mays on the Illumina 55K maize SNP array. All of these genotypes will be made publicly available upon publication, and will be linked to USDA GRIN germplasm records.

We have also generated two lists of candidate genes, based on the work of van Heerwaarden et al. (Submitted) and Hufford et al. (Submitted). We are already working with others to make use of these data, for example in understanding patterns of expression or selection on transcription factor binding sites. These lists of putatively selected genes will be published along with the papers describing the methods.

Training:

Lauren Sagara is an undergraduate student who has been trained in a number of laboratory techniques including germination, DNA extraction and quantification and PCR. She has performed the majority of the DNA preparation for genotyping of the lines used for the grant.

Matthew Hufford is a postdoctoral scholar who has developed the pipeline for germination, germplasm management, and DNA preparation. He has trained two undergraduates in laboratory methods, and has been learning computational methods for SNP analysis. He has led the analysis of 103 genomes of domesticated and wild maize to understand changes in selection during domestication and subsequent improvement (Hufford et al. Submitted).

Joost van Heerwaarden was a postdoctoral scholar who developed statistical approaches to correct for linkage (van Heerwaarden et al. 2010) and introgression (van Heewaarden et al 2011), and analyzed the chronological sampling of corn belt lines using a novel application of environmental association analysis (van Heerwaarden et al, Submitted). Joost has left the laboratory for a position at the University of Wageningen in the Netherlands.

Tanja Pyhäjärvi is a postdoctoral scholar, funded on an independent fellowship, who has been working on several projects related to the grant, including analysis of candidate genes (Hufford et al. Submitted) and association analysis of maize and teosinte genotypes with environmental variables and phenotypes (Pyhäjärvi et al. In prep).

Collaborations:

The collaboration with Rita Mumm has opened the opportunity to study in much greater detail the genetics of heterosis; with Dr. Mumm and others we will be sequencing her parental inbred lines and association genotypic differences with high-quality data from yield trials. Work with the maize diversity group, led by Ed Buckler, has allowed us to access to data from >100 maize and teosinte genomes to assess genome-wide patterns of selection in much finer genomic detail than we are able to do with our SNP data (Hufford et al. Submitted). A related collaboration with Dr. Nathan Springer has given us the opportunity to analyze expression along with alongside genotypic change at our candidate genes.

Evaluating Genomic Selection for Applied Plant Breeding

Kevin P. Smith

Project Director: Kevin P. Smith, University of Minnesota, smith376@umn.edu

Co-PDs: Jean-Luc Jannink, USDA-ARS, jeanluc.work@gmail.com

Clay Sneller, The Ohio State University, clay.sneller@gmail.com

Objectives and Accomplishments:

Objective 1: Compare the ability of genomic selection (GS) and phenotypic selection (PS) to predict future performance of lines and select the best lines.

In wheat, we have completed the second year of phenotyping a set of 470 wheat lines (progeny set) for yield (6 environments) and Fusarium head blight (FHB) index (2 environments) and one year of phenotyping for quality traits at two locations (second year's grain from two locations is being processed). We obtained a set of 1,820 DArT markers for 449 of the lines. We will soon genotype all 470 lines with SNP markers.

In barley, we have compiled historical phenotypic data from the progeny set (1204 lines) resulting from crosses involving 100 parents from the breeding program and calculated breeding values using common checks.

For both wheat and barley we have completed preliminary analysis of the efficiency of GS versus PS using a number of data sets for traits with a range of heritabilities (H). The phenotypic and genotypic data were used to obtain genomic estimated breeding values (GEBV) using ridge-regression in a cross-validation analysis. We obtained the correlation of the GEBV with the phenotypes (r) and then calculated the relative efficiency (RE) of one cycle of GS to PS as r/ $\sqrt{H}$. We have observed a range of RE values in these studies, but for many the value is near 0.5. A breeding cycle for PS is 4 and 7 years for barley and wheat respectively, and a breeding cycle for GS is one year. Therefore, GS would be 2 and 3.5 times as efficient per year compared to PS for barley and wheat, respectively.

Objective 2: Assess the ability of GS to predict a parent's breeding value

In wheat, we have completed the first year of phenotyping the parents of the 470 lines used in objective 1. We will submit these for SNP genotyping during the winter of 2011-12. The second year of field trials for these lines was planted in the Fall of 2011.

In barley, have completed data collection on the parent set which includes the 100 parents mentioned above and an additional 100 lines that are contemporaries of the parents for a total of 5 yield trials and 4 FHB trials. Analysis of grain samples for the 2010 trials are complete and 2011 samples are in progress.

Objective 3: Determine whether a trained GS model maintains accuracy over breeding cycles.

All the data has been assembled and this analysis is in progress. Preliminary results indicate that RE from cross-validation studies are generally higher than RE calculated from progenies of parents in the training population.

Objective 4: Use simulations to assess scenarios for the introduction and implementation of GS in a breeding program to optimize short- and long-term success over cycles of GS.

From long-term selection experiments in model systems, we know that mutation can play an important role in long-term response. However, GS will not adequately capture contributions from new mutations. We are currently setting up simulation experiments to assess what impact this issue might have on medium and long-term gain, and whether approaches can be developed to improve GS capture of mutational events.

Broad Impacts:

Based on results from simulation studies looking at long term gain, we implemented selection criteria that weights allelic effects based on frequency with the idea favorable alleles, currently at low frequency, will contribute to progress more in later cycles.

Deliverables:

Publications:

Lorenz, A. J., Chao, S., Asoro, F.G, Heffner, E.L., Hayashi, T., Iwata, H., Smith, K.P., Sorrells, M.E. and Jannink, J.L. 2011. Genomic Selection in Plant Breeding: Knowledge and Prospects. Advances in Agronomy 110:77-123.

Heslot N., Yang H.-P., Sorrells M.E., Jannink J.-L. 2011. Genomic Selection in Plant Breeding: A Comparison of Models, In Press. DOI: 10.2135/cropsci2011.06.0297.

Heffner E.L., Jannink J.-L., Iwata H., Souza E., Sorrells M.E. 2011. Genomic Selection Accuracy for Grain Quality Traits in Biparental Wheat Populations. Crop Science 51:2597-2606.

Oral/ Poster Presentations:

C Sneller, J-L Jannink, A. Hoffstetter, and A Cabrera. 2011. Preliminary evaluation of genomic selection for FHB resistance and other traits. 2011 Annual FHB Forum, St Louis, MO.

A. Hoffstetter, C. Sneller and A Cabrera. 2011. Association analysis of Fusarium Head Blight resistance in soft red winter wheat. 2011 Annual FHB Forum, St Louis, MO.

Smith, K.P, Lorenz, A., Jannink, J.L., Chao, S., Viram, V., Horsley, R. 2011. Genomic Selection for Fusarium Head Blight Resistance in Barley. 2011 Annual FHB Forum, St Louis, MO.

Training:

Amber Hoffstetter, MS graduate student (Objectives 1-2)

Antonio Cabrera, Post-doc (Objectives 1-2)

Ahmad Sallam, PhD graduate student (Objectives 2-3)

Hsiao-Pei Yang, Post-doc (Analyses in Obj. 1-3 and Obj. 4)

Nicolas Heslot, PhD graduate student (Model comparisons in Obj. 1)

Collaborations:

Dr. Sneller has received support to implement genomic selection in his breeding program from the Ohio Small Grains Marketing Program and from the US Wheat and Barley Scab Initiative to assess genomic selection in a set of 749 soft wheat genotypes.

Dr. Sneller has received a Fulbright Fellowship where he will focus on teaching many aspects of using whole genome scans in breeding.

Expanding the Scope of Association Mapping in Important Crop Species with Methodology Development in Statistics

Dong Wang

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Ismail Dweikat, University of Nebraska-Lincoln (idweikat2@unl.edu)

Kent M. Eskridge, University of Nebraska-Lincoln (keskridge1@unl.edu)

Objectives and Accomplishments:

Objective I (I). Develop statistical models capable of accommodating multiple QTLs, epistatic effects and GxE interactions using the adaptive mixed LASSO method.

Building on previous works, the application of adaptive mixed LASSO has been extended. Especially, we modified adaptive mixed LASSO for the setting of genomic selection. A more relaxed criterion has been developed to achieve good prediction properties. Both simulation studies and applications to the Nebraska wheat breeding data as well as data sets from CIMMYT have shown that the proposed method has excellent potential in improving plant breeding practices.

Objective (II). Establish a mapping population using accessions in the Nebraska Wheat Breeding Program and carry out association analysis on a variety of traits.

In addition to 280 accessions processed in 2010, phenotype and genotype data have been collected on 280 additional wheat accessions in the Nebraska Wheat Breeding Program during the year 2011. Grain yield, test weight, height, heading date and disease resistance were measured at up to nine locations in Nebraska. These accessions were genotyped with DArT markers at Diversity Array Technology Pty. Ltd. More than 1000 DArT markers are found to be of high quality and polymorphic. The analysis of these data is ongoing and several QTLs have been detected.

Objective (III). Develop software to facilitate the application of the proposed methods by plant researchers.

With most of coding finished, we are in the process of putting together a R library for release. Responding to request, some code has already been provided to other researchers. Features for recently developed methodology related to genomic selection will be incorporated.

Broad Impacts:

This project has stimulated broad interest in association mapping and genomic selection in the life science community of University of Nebraska. The PD and co-PDs have discussed with other researchers on potential collaborations in related efforts. Furthermore, this opens opportunity for collaborating with researchers in plant genetics at other institutions. This project has also helped to create a joint agronomy/statistics Ph.D. program between the two departments.

Deliverables:

Publications:

Wang D, Eskridge KM and Crossa J. 2011. Identifying QTLs and Epistasis in Structured Plant Populations Using Adaptive Mixed LASSO. ***Journal of Agricultural, Biological, and Environmental Statistics***, 16: 170-184.

Wang D, El-Basyoni IS, Baenziger PS, Crossa J, Eskridge KM, and Dweikat I. 2011. Prediction of Genetic Values of Quantitative Traits with Epistatic Effects in Plant Breeding Populations. Under revision.

Oral/ Poster Presentations:

Wang D. Modeling Complex Traits in Plant Association Studies, a Shrinkage Based Approach. International Plant and Animal Genome Conference XIV, San Diego, CA, January 2011.

Community Resources Generated:

Genotype data with more than 1000 DArT markers and phenotype measurements on multiple traits are available on 560 wheat accessions. Prepublication access to these materials are available from the PD and co-PDs.

Training:

Ibrahim Salah El-Baysoni, graduate student in Department of Agronomy and Horticulture, University of Nebraska-Lincoln. He carried out the phenotyping and genotyping of wheat accessions. He has also identified several potential QTLs from this population.

Wei Liu, graduate student in Department of Statistics, University of Nebraska-Lincoln. She carried out development of a new computation algorithm that is potentially much faster and scalable.

Collaborations:

Dr. Jose Crossa of International Maize and Wheat Improvement Center (CIMMYT) and Dr. Jiankang Wang of Chinese Academy of Agricultural Sciences have collaborated with us on association mapping and genomic selection.

Defense versus Symbiosis: Host Genetic Control of Nodulation Specificity in Soybeans

Hongyan Zhu

Project Director: Hongyan Zhu, University of Kentucky, hzhu4@uky.edu

Objectives and Accomplishments: Leguminous plants can enter into root nodule symbioses with nitrogen-fixing soil bacteria known as rhizobia. An intriguing but still poorly understood property of the symbiosis is its host specificity, which is controlled at multiple levels involving both rhizobial and host genes. The goal of this project was to clone the two soybean genes *Rj2* and *Rfg1* that restrict nodulation with specific strains of *Bradyrhizobium japonicum* and *Sinorhizobium fredii*, respectively.

We have successfully cloned the two genes. We showed that *Rj2* and *Rfg1* are allelic genes encoding a member of the Toll-interleukin receptor/nucleotide-binding site/leucine-rich repeat (TIR-NBS-LRR) class of plant resistance (R) proteins. Our discovery is consistent with recent reports describing rhizobial T3SS and its secreted effectors that play an important role in modulation of host range, and suggests that establishment of a root nodule symbiosis requires the evasion of plant immune responses triggered by rhizobial effectors. This finding may also offer novel strategies to enhance symbiotic nitrogen fixation in crop legumes. For example, the nodulation-restrictive *R* genes may be manipulated so that a host can deterministically interact with rhizobial inoculants with high nitrogen-fixing efficiency and exclude those indigenous strains that are highly competitive but with very low nitrogen-fixing efficiency. We are also in the process of cloning another dominant soybean gene, called *Rj4*, which restricts nodulation with *B. elkanii* USDA61. Intriguingly *Rj4* is not an *R* gene, and we believe that the result from this research will have significant impact in the area of plant-microbe interactions.

Deliverables:

Publications:

Yang S, Tang F, Gao M, Krishnan HB, Zhu H (2010) *R* gene-controlled host specificity in the legume-rhizobia symbiosis. *Proc Natl Acad Sci USA*. 107(43):18735-40.

Wang D, Yang S. Tang F, Zhu H (2011) Symbiosis specificity in the legume-rhizobial mutualism. *Cellular Microbiology* (in press).

Oral Presentations: *R* gene-controlled host specificity in the legume-rhizobia symbiosis. Model Legume Congress 2011, France

Training: Shengming Yang (postdoc) and Fang Tang (graduate student)

Collaborations: Dr. Hari B. Krishnan, US Department of Agriculture–Agricultural Research Service and Division of Plant Sciences, University of Missouri, Columbia, MO 65211; Dr. Jeff Chang, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331; Dr. Dong Wang**,** Department of Biochemistry and Molecular Biology**,** University of Massachusetts-Amherst, Amherst, MA 01003.

Plant Breeding and Education Program Project Reports

(Alphabetical Order of Lead Project Director)

Assessing the Cost of Pyramiding Host Resistance to Biotic Stress in Crop Species

William Berzonsky

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Project website: D2L login page: https://d2l.sdbor.edu/
Username: **sdsuguest.1** and Password: **sdsuguest.1** Once logged in, click on "Guest Observer"

Objectives and Accomplishments: The education objective is to develop a course which optimizes the graduate student experience in both laboratory genetic and field-based breeding methodology. The purpose of this experience is to adequately educate and train successful "next-generation" plant breeders for potential employment in private industry and academia. The research objective is to assess the cost of resistance to leaf rust in sunflower and wheat, particularly under drought stress conditions.

Broad Impacts: In the fall 2011 semester, Plant Breeding Techniques (PS741), which was established to achieve the education objective was taught for the second time. As a graduate level course, it had another relatively large enrollment of 11 students. An unanticipated outcome is that two students are undergraduates. Once again, students were able to interact with breeders and staff personnel working with both cross and self-pollinated crops such as corn, soybean, sunflower and wheat (**Fig. 1**). However, compared to last year and as a consequence of visiting Pioneer and Monsanto corn and soybean breeding programs, students were exposed to a more balanced perspective on public and private breeding programs. Examples of breeding programs and plant breeders who interacted with PS741 students include; the NDSU Corn Breeding Program (Fargo, ND), the USDA-ARS Sunflower Breeding Program (Fargo, ND), the Pioneer Soybean Breeding Program (Volga, SD) and the Monsanto Corn and Soybean Breeding Programs (Harrisburg, SD). An outcome of this experiential learning is that students began to comprehend intricacies of breeding programs that are otherwise not available in textbooks.

Because they were able to compare their experiences between public and private breeding programs, students recognized important differences. As an example, students learned that there is substantial collaboration between all soybean breeding programs within Pioneer, regardless of their physical proximity. The strength of this approach is that the interchange of germplasm enables the company to maintain a broader genetic base, which consequently helps sustain genetic gain. A major difference recognized between public and private breeding programs is that private programs follow a rigid schedule of standard operating procedures compared with public programs. Such rigidity results in the most efficient use of resources, but it also limits the ability of the breeder to rapidly change in response to emerging producer problems.

Hybridizations were made to pyramid host leaf rust genes in both wheat and sunflower. A total of 164 crosses putatively combining *Lr10* and *Lr21*, 191 crosses putatively combining *Lr10* and *Lr34*, and 201 crosses putatively combining *Lr21* and *Lr34* are complete for wheat. A graduate student is continuing to validate the pyramided combinations in wheat using the appropriate *Lr* gene markers. Plans are to utilize allopurinol, an inhibitor of the hyposensitive leaf rust reaction, to help assess the cost of expressing host resistance genes in the near-isogenic wheat lines and eventual pyramided leaf rust gene combinations in sunflower and wheat. Five sunflower hybrids and three backcrosses were produced, and crosses are expected to carry *R1*, *R4b*, or *R5* for resistance to leaf rust.

Figure 1. (Left) PS 741 students interact with North Dakota State University corn breeder, Dr. Marcelo Carena, **(Middle and Right)** students visit Pioneer and Monsanto breeding facilities in Volga and Harrisburg, SD, respectively.

Deliverables :

Oral/ Poster Presentations:

Berzonsky, W.A., K.D. Glover, K. Grady, J.L. Gonzalez-Hernandez, and J.M. Stein. 2010. Assessing the cost of pyramiding host resistance to biotic stress in crop species. Plant and Animal Genome Meetings, SD, CA (Jan. 15-19, 2011).

Community Resources Generated: Sunflower and wheat germplasm lines with unique combinations of leaf rust resistance genes will be available to breeders as the lines are produced and the gene combinations are verified.

Training:

Christine Lubenow, advisee to Dr. William Berzonsky is receiving training and developing skills in plant pathology, plant breeding, and molecular genetics. Thumbiko Mkandiwire, advisee to Dr. Karl Gover is receiving training and developing skills in plant pathology, plant breeding, and molecular genetics. Eleven (undergraduates, MS, and PhD candidates) enrolled in PS741 are learning hybridization techniques (for cross and self-pollinated crops), molecular techniques as applied to breeding, and other plant breeding techniques.

Collaborations:

Expanding PS741 to involve more private plant breeding programs produced more interactions with industry, specifically Pioneer International and Monsanto breeding programs throughout South Dakota. The teaching collaboration was strengthened between breeders of cross and self-pollinated crops at North Dakota and South Dakota State Universities as well as the USDA-ARS. The project advisory panel, consisting of breeders from public institutions and representatives from Monsanto and Syngenta convened, and the panel made specific suggestions to the project PI for strengthening project teaching and research components.

Translational Genomic Approaches for Enhancing Disease Resistance in Plants, an Internet-Facilitated Education Program for Training Plant Breeders

Charles Brummer

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Project website: http://projects.coe.uga.edu/plantbreeding/

Objectives and Accomplishments:

An internet-based plant breeding e-textbook.

The wiki book is setup at the website above. We are still in process of editing content and developing graphics before this goes live. The courses that form the basis of this e-text have been taught in spring 2011 (Advanced Plant Breeding, which was taught by Dr. Brummer from Oklahoma using Skype) and in fall 2011 (Introductory Plant Breeding, taught by Dr. McGregor). In both cases, graduate students developed papers on topics that will be included in the textbook. We are setting up eLearning course modules for translational breeding with modules in various states from storyboards to draft versions. These will be completed by the end of 2012. The primary effort at the current time is the writing and editing of content, and the integration of graphics and other media.

A recruitment lecture and game to attract students to plant breeding careers.

Developed an introductory plant breeding lecture (50min) to recruit students. The lecture starts with the history of Plant Breeding, including the development of hybrid corn and the green revolution and also current plant breeding methods such as the use of GMOs and MAS. This lecture was given in HORT 2000 (Horticultural Science) in Spring and Fall 2010. The HORT 2000 class is taught in 2 sections with between 200 and 300 students per section per semester. I estimate the lecture reached approximately 800 students (assuming everybody attended!). We have set up a game which will play on the web and on the iPad. It has been designed and a prototype built. This will be uploaded to the website when it has been debugged and found to be stable.

Research to develop and deploy SNP markers in soybean, watermelon, and peanut.

Breeder-friendly molecular markers (SSR and CAPS) have been developed for nematode resistance in peanut and for several traits in soybean. High throughput breeding line evaluation for marker-based selection in the soybean breeding program is ongoing using breeder-friendly markers on a LightCycler. Markers have been used to combine nematode resistance with high oleic:linoleic acid ratio in peanut with the latter being selected using a high-throughput (LightCycler), breeder-friendly SNP marker. The same population segregating for nematode resistance is being screened for leaf spot resistance to allow QTL mapping using SSR and SNP markers. A GoldenGate array for peanut has been constructed and was shown to detect polymorphisms among inbred peanut lines including parents of the two mapping populations segregating for leaf spot and TSWV resistance. The GoldenGate assay detects homeologous loci in tetraploid peanut; therefore, calls of heterozygous and one homozygous class are most common, although the heterozygous class does not reflect true heterozygotes. Implementation of marker-assisted backcrossing in the peanut breeding program has resulted in the development of a high-oleic, nematode resistant line whose release as a cultivar is anticipated. Training of peanut breeders on the application of molecular markers in peanut was conducted through a workshop. In watermelon, the

parents of the 2 mapping populations were screened for gummy stem blight resistance in the greenhouse. One of the parents (Delagoa) of the elite x citron population showed good resistance. The $F_{2:3}$ seeds for the elite x citron population were planted in the greenhouse to develop RILs for replicated field screening. The $F_{2:4}$ seeds are currently being harvested and planted for further advancement.

Broad Impacts:

Our e-book, which is under development, will be freely accessible world-wide, providing plant breeding students anywhere with up-to-date information regarding plant breeding methods.

Deliverables:

Publications:

Chu, Y., C.L. Wu, C.C. Holbrook, B. L. Tillman, G. Person, and P. Ozias-Akins. 2011. Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. The Plant Genome 4:110-117.

Training:

Dan Tinsley (undergraduate – Brummer) – capturing video and photos of various plant breeding programs

Vickie Waters and Kiranjit Kaur (PhD student – McGregor) – watermelon mapping and SNP development

Ye Chu and Rattandeep Gill (MS student – Ozias-Akins) – peanut SNP mapping

Ananta Acharya (PhD student – Brummer) – recruitment module and game

Daisyane Barreto, Cole Sherer, Erkan Er, Gulgun Afacan, Tony Gonzalez, Milan Anich (Orey) – layout, graphics, programming for e-book, eLearning modules, and recruitment game

Hussein Abdel-Haleem, Jennie Alvernez, and Bo-Kuen Ha (Boerma) – developed and taught the hands-on translational breeding workshop; developed materials for the e-learning translational genomics module

Collaborations:

We have developed a very strong collaboration between several plant breeders (Brummer, Boerma, Ozias-Akins, and McGregor) with Dr. Mike Orey in the College of Education.

Enhancing Education and Research Capacity in Plant Breeding for Drought Tolerance

Pat Byrne

Project Director: Pat Byrne, Colorado State University (CSU) Patrick.Byrne@colostate.edu

Co-PDs:

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Stephen Baenziger	University of Nebraska-Lincoln (UNL)	pbaenziger1@unl.edu
Bill Bauerle	Colorado State University	Bill.Bauerle@colostate.edu

Project website: http://www.droughtadaptation.org/

Objectives and Accomplishments:

The project's educational objectives are to (1) develop and offer an online course and a field-oriented short course on the genetics, breeding, and physiology of drought stress tolerance; (2) train one Ph.D. and two M.S. students for leadership roles in plant breeding for drought tolerance; and (3) expand research opportunities for undergraduate students.

Accomplishments: (1) The graduate level, 1-credit online course was held in Fall, 2011 with an enrollment of 23 students. The course was very favorably evaluated and will be offered again in Fall, 2012. The next two-week short course has been scheduled for June 11-22, 2012 at CSU in Fort Collins. The course will carry 3 graduate level credits and has a capacity of 25 students. (2) An M.S. student at UNL completed her degree in May, 2011. Another M.S. student at UNL and a Ph.D. student at CSU continued their graduate programs. (3) Two undergraduate students conducted research projects on traits related to drought tolerance: coleoptile length and root characteristics of wheat seedlings.

The project's research goal is to improve drought tolerance in winter wheat by incorporating synthetic hexaploid wheat germplasm. Specific objectives are (1) to assess yield performance under wet and dry conditions of wheat populations derived by backcrossing synthetic hexaploids to the adapted cultivars 'Hatcher' and 'Goodstreak'; (2) to evaluate physiological traits in these populations and determine their relationship with yield components under drought stress; (3) to determine chromosome segments of the synthetics that are preferentially retained in the most drought tolerant materials.

Accomplishments: Cultivar x synthetic hexaploid backcross populations were evaluated in a total of eight field trials in Nebraska and Colorado in 2009-2010 and 2010-11. Several families within the synthetic x Hatcher crosses had mean yields across eight environments greater than Hatcher, indicating that although the synthetic hexaploids are unadapted to Great Plains conditions, they contain useful yield-enhancing alleles. Results for the synthetic x Goodstreak crosses were not as promising, though some of the crosses approached the yield level of Goodstreak. Selected heads from the best subset of families

were planted in head-rows in Fall, 2011 and the best 10% will be selected in summer of 2012. Greenhouse studies have evaluated physiological traits and root morphological differences among the parental lines and F_1 progeny.

Broad Impacts:

Development of the online course on plant breeding for drought tolerance completes the second major instructional component of this project. We now have in place a two-week field-oriented, resident instruction course, for students who are able to devote the time and funding to that type of course, and an online distance education course for students who prefer or require an alternative mode of learning. To date 50 students have participated in these courses, which will both be offered again in 2012.

Deliverables:

Publications:

K. Onweller. 2011. Disease and Insect Resistance and Quality Characterization of Six CIMMYT Synthetic Hexaploid Wheats. M.S. Thesis, University of Nebraska-Lincoln.

Oral/ Poster Presentations:

Becker, S. 2011. Drought Tolerant Trait Identification In Synthetic Hexaploid Wheat. Western Society of Crop Sciences annual meeting, Laramie, WY, June 20-21, 2011. Abstract 69060: http://a-c-s.confex.com/crops/ws2011/webprogram/Paper69060.html.

Becker, S., Byrne, P.F., Reid, S.D., and Bauerle, W.L. Drought Tolerant Trait Identification in Synthetic Hexaploid Wheat. Crop Science Society of America Annual Meeting, Oct. 16-19, 2011, San Antonio, TX. Abstract 6498: http://a-c-s.confex.com/crops/2011am/webprogram/Paper64981.html.

Community Resources Generated:

Ten backcross wheat populations were developed from the crosses of the adapted wheat cultivars Hatcher and Goodstreak with each of five synthetic hexaploid lines. Limited quantities of seed are available by request to Drs. Byrne or Baenziger.

Other products/ outcomes:

SOCR 580A2, Plant Breeding for Drought Tolerance, an online course offered through Colorado State University Distance Education (Online Plus).

Training:

CSU: Twenty-three students from public and private sector institutions participated in the online course. Graduate students Annie Heiliger and Steve Becker obtained teaching experience in online education. As part of his Ph.D. research, Becker received training in evaluating physiological and root traits. Four undergraduate students helped with field and greenhouse evaluations.

UNL: Kayse Onweller successfully defended her M.S. degree, which involved screening the parents of the populations for resistance to multiple diseases and insects. M.S. student Russell Ward joined the project and is studying the inheritance of stem rust resistance identified in two of the synthetic parent lines. Goodstreak is resistant to stem rust, but a potentially new gene coming from the synthetic wheat lines could be very valuable in our efforts to create gene pyramids that are resistant against new virulent races of stem rust coming out of Africa. This year six undergraduate students assisted with the field aspects of the program and four undergrads helped with the greenhouse project.

Building Expertise in Plant Breeding that Focuses on Drought Tolerance in Common Bean (Phaseolus vulgaris L.)

James D. Kelly

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Project website: http://www.css.msu.edu/bean/ MSU Dry Bean Breeding and Genetics Program http://worldtap.msu.edu/short-courses/mpb/ Molecular Plant Breeding short course offered through the World Technology Access Program (WorldTAP) at MSU.

Objectives and Accomplishments:

1) Develop expanded greenhouse phenotypic screening protocols for drought resistance in common bean.

Accomplishments: Eight dry bean genotypes with known tolerance to drought in the field were evaluated in small 9 cm^2 pots in the greenhouse to measure recovery from a 2-week period without watering. The root was constrained in this system to investigate shoot mechanisms underlying drought resistance in bean seedlings. Parameters measured were wilting, unifoliate senescence, stem greenhouse, recovery after resuming watering, plant biomass, pod and seed numbers. Certain cultivars did not show any significant difference in pod number under both drought stress and root restrictions, whereas others showed significant reduction in pod numbers, suggesting that they rely more on root traits to sustain them through prolonged periods of drought in the field.

In other root growth restriction experiments, four different dry bean varieties grown in similar pots in growth chambers were also measured for photosynthesis and conductance rates as determined via gas exchange measurements. Three-week old plants were either kept in a well-watered condition or exposed to drought stress by withholding water, and measurements were taken when plants reached a set pot weight. Differences in photosynthesis among the four varieties were found in both well-watered and drought treatments. Higher rates of photosynthesis during drought correlated with greater above ground biomass dry weight. Differences in photosynthesis were further dissected by determining light-response and A-Ci curves on the four varieties under drought stress, with significant differences found under drought conditions.

2) Identify QTLs associated with drought resistance in common bean and then use them to develop highly drought resistant bean lines with good agronomical characteristics. Accomplishments: A 125-entry RIL population from the cross of SEA5 x CAL96 was evaluated under stress and non-stress at Karama, Rwanda for two growing seasons (Nov 2010 to March 2011 and April to July 2011). Geometric yields ranged from 428 to 2342 kg/ha and phenological and harvest index data were also collected for use in QTL analysis. The same RIL population is being genotyped at MSU with Simple Sequence repeat (SSR) markers with the goal of mapping QTL associated with drought resistance in beans. Parents were screened with 460 SSR markers of which 148 were polymorphic representing a polymorphism level of 32 % between the parents. The genotyping of the entire mapping population is being conducted. The population has already been evaluated with74 polymorphic primers.

Ninety-six bean genotypes from the BeanCAP project (http://www.beancap.org/) specifically selected for drought evaluation, were grown in the field during the summer 2011 season at the Montcalm Research Farm in Michigan. The site was selected to create drought as the soil type is very coarse textured McBride sandy loam. Plots were not supplied with any supplementary irrigation and experienced early season drought through late July. Genotypes were planted in 6m rows, 0.5m apart. When fifty percent of the genotypes were flowering, five plants from each plot were excavated and data was recorded for: basal root angles, basal root branching in a three centimeter segment, number of basal roots, number of adventitious roots, adventitious root branching in a three centimeter segment, tap root diameter, tap root branching in a three centimeter segment, an overall root score, and dry weight of shoots and roots. We followed the 'shovelomics' protocol described at Penn State web site (http://roots.psu.edu/en/node/945).

At the end of the season, plots were trimmed to 5m, plants were mechanically pulled using Pickett and total plant biomass was recorded before threshing. Throughout the growing season, other data taken included: flowering date, maturity date, lodging, height, seed weight and yield. The root data will be compared with performance data for MI but from five other locations in the US where the same BeanCAP trial was grown under drought stress.

3) Further develop a stable transformation system for common bean.

Accomplishments: Following last year's transient transformation studies on common bean, we finalized the conditions for efficient gene delivery using different *Agrobacterium tumefaciens* strains. The newly

optimized conditions enabled all inoculated explants to show strong transient GUS expression. To further optimize our regeneration systems, two basal media (MS and WPM) and two types of embryo explants were evaluated. The physiological status of initial embryo explants, which were induced on different media, showed significant impact on subsequent regeneration. An improved regeneration system with a novel regeneration pattern has been developed. Following last year's stable transformation studies, one stable transformant with GUS-positive roots and leaves were obtained; however, this transformant did not eventually develop into a phenotypically normal plant due probably to the chimeric nature or the effect of insertion position. Using the optimized regeneration and gene delivery systems, six stable transformations, each with 200-300 embryo explants, were conducted in August, 2011 using either 'Merlot' or 'Red Hawk' cultivars. After about three months selection on regeneration medium, 5-10% explants produced tiny green buds or shoots. While this is significant progress, more effort is still needed to verify the reliability and reproducibility of this new system. More transformations using the herbicide resistant *bar* gene have been planned for next year.

4) Offer a new course Molecular Plant Breeding (MPB) targeted at graduate students and international scientists interested in plant breeding program at MSU.

Accomplishments: The short course was offered for the third time from August 21-26, 2011. Topics covered in the course are listed on the web site. In addition to these topics, a half day field trip was arranged to visit the potato, soybean and bean breeding programs at MSU where participants received an appreciation of the number of different traits that plant breeders need to consider along with biotic and abiotic stress tolerant traits that contribute to a successful variety. This course was attended by 13 participants representing ten countries (China, Colombia, Ethiopia, Ghana, India, Sri Lanka, Taiwan, Tanzania, Uganda, and USA). Participants of the course were from various backgrounds including three graduate students of the Plant Breeding, Genetics and Biotechnology (PBGB) program, visiting scholars and international participants. Among the group were six professional plant breeders (Rice, Tef, Cassava, Soybean, Okra and Pea) from Asia and Africa. A special session was arranged for the participants to make a short 10 minutes presentation on the use of molecular markers in their breeding program/research in their home countries/at MSU. The course benefited greatly by having many plant breeders as participants in the course who had previously not used molecular marker approaches in their breeding programs but, were interested in doing so. The course evaluation recoded an average rating of 9.5 out of 10 for the question whether the course met the expectations of the participants.

Broad Impacts: Michigan is the second largest producer of dry bean in the U.S. Over 90% of bean acreage is grown under rainfed conditions in the Great Lakes watershed. Finding bean germplasm and varieties with enhanced level of resistance to drought is critical to sustain the bean industry in the state as summer rainfall patterns over the last decade continue to diminish in quantity and regional distribution. Bean genotypes with improved water use efficient would be valuable in irrigated areas in the western US where water costs are increasing and its availability for irrigation is more restrictive.

Deliverables:

Oral/ Poster Presentations:

J.D. Kelly presented invited paper: Strategies to improve adaptation of common bean to drought at the Biennial Meeting of Bean Improvement Cooperative, San Juan PR. Oct 31- Nov 2, 2011.

Gerardine Mukeshimana presented at poster on: Phenotypic evaluation of dry bean RIL population for drought resistance in Rwanda, at the same meeting.

Community Resources Generated: Bean RIL populations developed by the project have been used by colleagues at Penn State to study effect of root architecture and depth in drought prone soils deficient in phosphorous. A second RIL population developed in large red mottled seed type was sent to Rwanda for testing under drought conditions.

Training: Graduate student Valerio Hoyos joined the program as a doctoral candidate in 2011 and Valerio participated in MBP short course. He will evaluate genotypes from the BeanCAP for reaction to drought. Three undergraduate students, Riley Marshall, Mary Marshall and Cindy Amstutz assisted in the program, conducting bean hybridization, DNA extraction, running SCAR and RAPD markers, and disease screening.

Collaborations: Exploring possible new funding opportunities to work on drought stress in bean in collaboration with Dr. Steve Beebe at CIAT, Colombia to further drought research in Rwanda. In addition we have had discussions with Dr. Liz Van Volkenburgh at the University of Washington on ways to collaborate on research on photosynthesis of beans under drought stress. Student research projects will involve study of photosynthetic traits under stress; and evaluating bean genotypes from the BeanCAP project for drought and determination of additional physiological parameters that could be used in field screening for drought.

Advanced Pine Breeding Through Association Genetics and Biotechnology

Matias Kirst

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Project website: www.sfrc.ufl.edu/forestgenomics/

Objectives and Accomplishments:

The **research** objectives of this project are to perform deep allele sampling in association populations of loblolly pine, to identify and verify causative alleles of large effect on trait phenotypic variation. Originally, deep allele discovery, genotyping and association genetic analysis was planned for a set of 40 genes, for which significant associations with biomass growth, wood quality, disease resistance and drought tolerance had been identified by us previously. This aim would be pursued by expanding the sequence beyond previously characterized regions, and re-sequencing. However, availability of sequence capture methods has allowed us to expand this goal to over 10,000 genes. Initially a set of probes designed to capture 6.6Mbp of the 21.7Gbp loblolly pine genome was tested, and capture efficiency was shown to exceed 70%. Currently, capture of target genes has been largely completed in an association population of 900 genotypes, and sequencing is under way. We also expanded the use of this approach to (1) characterize the genetic diversity of the 10,000 genes in a set of 24 unrelated individuals of loblolly and slash pine that represent the diversity of the two species, and (2) map the majority of the genes by analyzing a segregating population. In the second objective of this project we will functionally test associations with gain- and loss-of-function alleles introduced by genetic transformation to verify the expected phenotypic effect, in collaboration with a forest tree seed company (ArborGen, LLC). Finally, existing genotypic data available for the association population is being used to evaluate and apply genomic selection to accelerate breeding of conifers. Our data indicates that one of our association populations is a suitable training set, from which we now developed accurate prediction models for early selection and mate allocation in breeding programs. Using information from predictive models we have initiated crosses designed to generate families that are highly productive in biomass, disease resistant and broadly adapted. This approach can reduce the length of breeding cycle 15+ to less than 5 years.

Our **education** objectives of this project are to train graduate students in a customized curriculum with courses in genetics and breeding, molecular biology and field-based physiological genetics. In that effort we have recruited four Ph.D. students who are active participants in this project. These students began their degree in the Fall of 2010, and are currently taking courses outlined in the training plan proposed in the project, and actively participating in project's research.

Broad Impacts:

The sequence capture approach employed in this project is allowing us to greatly expand sequence knowledge beyond EST contigs – one of the original goals of the project. However, this goal is being achieved for a set of genes 2-3 orders of magnitude higher than originally proposed. In a parallel study, the use of sequence capture for discovery and genotyping of polymorphisms in slash pine, a related species to loblolly pine, will allow us to identify genes under natural selection and that may be involved in adaptation. Finally, this approach is also being applied to map loblolly pine genes – currently 2,500 have been positioned and work is in progress to locate the remaining. Finally, expanding our focus of

research to the use of genomic selection in pine breeding, based on genotypic data generated in the funded project, is creating new uses for the data to accelerate breeding.

Deliverables:

Publications:

Resende MF Jr., Muñoz P, Acosta JJ, Peter GF, Davis JM, Grattapaglia D, Resende MD, Kirst M. (2011) Accelerating the domestication of trees using genomic selection: accuracy of prediction models across ages and environments. New Phytologist doi: 10.1111/j.1469-8137.2011.03895.x.

Harfouche A, Meilan R, Kirst M, Morgante M, Boerjan W, Sabatti M, Mugnozza GS. Accelerating the domestication of forest trees. Trends in Plant Science (*in press*).

Resende, MFR Jr, Muñoz, P, Resende MDV, Garrick DJ, Fernando RL, Davis JM, Jokela EJ, Martin TA, Peter GF, Kirst M. Accuracy of genomic selection methods in a standard dataset of loblolly pine (Pinus taeda L.). Genetics (*submitted*).

Oral/ Poster Presentations:

Resende Jr., M.; Jaramillo, J.J.A.; Resende, M.D.V. and M. Kirst (2011) Genomic selection and next-generation genotyping to hyper-accelerate pine breeding. Plant and Animal Genome Conference XIX, San Diego, CA.

Neves, L.G.; Chamala, S.; Davis, J.M., Barbazuk, W.B. and M. Kirst (2011) Whole-exome sequencing and genotyping in the loblolly pine (Pinus taeda) megagenome. Plant and Animal Genome Conference XIX, San Diego, CA.

Neves, L.G. (2011) Targeted sequencing in the loblolly pine (Pinus taeda) megagenome by exome capture. 24th Annual PMCB Workshop, Daytona, FL.

Neves, L.G.; Davis, J.M; Barbazuk, W.B. and M. Kirst (2011) Targeted sequencing in the loblolly pine (Pinus taeda) megagenome by exome capture. IUFRO Tree Biotechnology 2011 Conference, Arraial D'Ajuda, Bahia, Brazil.

Resende Jr., M.; Munoz Del Valle, P.R.; Acosta, J.J.; Resende, M.D.V; Grattapaglia, D.; Kirst, M. (2011) Stability of Genomic Selection prediction models across ages and environments. IUFRO Tree Biotechnology 2011 Conference, Arraial D'Ajuda, Bahia, Brazil.

Neves, L.G.; Chamala, S.; Davis, J.M; Barbazuk, W.B. and M. Kirst (2011) Exploring the megagenome of Pine by targeted resequencing. Florida Genetics 2011, Gainesville, FL.

Resende Jr., M.; Neves L.G., Balmant K.M., Dervinis C., Nazarian A., Riva A., Kirst M. (2011) RAPID-Seq: A method for genome complexity reduction and high throughput genotyping. Florida Genetics 2011, Gainesville, FL.

Acosta JJ, Neves LG, Davis JM, Kirst M (2011) Genome-wide genetic diversity analysis of two *Pinus* species. Florida Genetics 2011, Gainesville, FL.

Community Resources Generated:

1. Set of oligonucleotide probes that are efficient for sequence-capture a large fraction of the coding sequence of ~14,000 pine genes.
2. Mapping of ~10,000 loblolly pine genes (in progress).
3. Genetic diversity data for those genes, based on the sequence analysis of 24 individuals that encompass the natural range of loblolly pine.
4. Genomic Selection prediction models for early selection of progeny derived from crosses in the association population.

Training:

The training of four Ph.D. level students has or is being currently funded through this project, supplemented by matching assistantships from the University of Florida.

Enhancing Education and Research in Breeding for Plant Disease Resistance

Michael Mazourek

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Co-PDs:

Walter De Jong, Cornell University, wsd2@cornell.edu

Susan McCouch, Cornell University, srm4@cornell.edu

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Martha Mutschler, Cornell University, mam13@cornell.edu

Rebecca Nelson, Cornell University, rjn7@cornell.edu

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Project Website: http://plbrgen.cals.cornell.edu/afri

Objectives and Accomplishments:

Plant breeding for disease resistance is a continual process because pathogens are remarkable for their ability to adapt and evolve to overcome plant resistances. New generations of plant breeders who can utilize state-of-the-art tools and resources must be trained to continue to respond to these needs, protect national food security and agricultural profitability. Our objectives were designed to respond to this need by enhancing our training of graduate and undergraduate students by creating research opportunities that combine bioinformatics, fieldwork, exposure to horticultural and agronomic crops, and by supplementing our curriculum. To extend these resources more broadly professionally produced Learning Modules were created that can be distributed on various media.

Broad Impacts:

We have linked together multiple facets of plant breeding for disease resistance toward creating the next generation of plant breeder that is prepared to use modern tools and approaches and integrate a broad range of perspectives.

Deliverables:

Oral/ Poster Presentations:

The following additional lectures were recorded to complete our Learning Modules:

"Genetics of Disease Resistance and Adaptation in Maize" Randall Wisser, Assistant Professor, Department of Plant and Soil Sciences, University of Delaware

"Breeding for pest/disease resistance in potato" Walter De Jong, Associate Professor Plant Breeding and Genetics, Cornell University

"Resistance to plant diseases in an agricultural context" Rebecca Nelson, Professor, Plant Breeding and Genetics, Cornell University

Community Resources Generated:

SGN and Gramene datasets were further developed.

Training:

William Holdsworth and Anna Levina are PhD student trainees on this project. Bill joined the Mazourek lab after rotations with the Mazourek, Nelson and Sorrells groups. Bill's projects include breeding for oomycete resistance, genotype by sequencing in vegetables and developing SNP markers for gene pyramiding in the Mazourek group breeding program. Anna joined the De Jong lab after three rotations including the De Jong, Sorrells and Mazourek groups. Anna's projects leverage resources developed by the SolCAP project. She had genotyped a tetraploid potato population with 8,000-plus SNP markers and

is now working to map loci that confer resistance to common scab. Both students are engaged in both applied breeding programs that release cultivars and utilizing modern genomics.

Elena Olsen and Katherine Scheibel are undergraduate students that served as student TAs for Plant Genetics PLBR2250. Katherine designed and led a lab activity focused on virus resistance in squash. Elena worked on the development of a lab exercise exploring insect resistance in Arabidopsis.

Samantha Klasfeld was a Cornell undergraduate summer intern in the Mazourek group. In addition to helping in the field, she assembled a panel of pepper germplasm with contrasting disease resistance and quality traits and co-developed and tested SNP markers linked to those traits. Markers that perform well in diverse backgrounds are highly useful for pyramiding simply inherited traits with *Phytophthora capsici* resistance in pepper.

Undergraduate students worked with the Sol Genomics Network and Gramene. Benjamin Gordon worked at SGN (http://solgenomics.net) curating Solanaceae data for loading into the database, including Solanaceae pathogens. He also wrote Perl scripts to organize the data. Zach Hempstead and Cindy Chen assisted with bioinformatics and programming projects, using Perl and PHP to complete a variety of objectives related to the Gramene project. Programs developed functionality for interacting with relational databases, parsing biological data and creating user interfaces for biologists.

A pair of undergraduate students from Texas A&M, Kingsville were hosted as summer interns. Paulo Garcia Jr worked with the Nelson group and in addition to being involved in daily activities managing a maize disease resistance nursery, Paulo individually spearheaded a new project aimed at identifying maize endophytes via the availability of abundant data from the Buckler lab on campus. Omar Vasquez worked with the Mutschler group and also participated in the field program planting and maintaining fields and collecting phenotypic data. Omar was individually responsible for the genotyping of tomato breeding lines to confirm the presence of introgressions.

Collaborations: All of the PDs have numerous existing collaborations

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New Vistas in Plant Breeding Education: Investigating the Genetics Underlying the Ability to Yield Under High Planting Density in Maize

Rita H. Mumm

Project Director: Rita H. Mumm, University of Illinois, ritamumm@illinois.edu

Co-PDs: Martin O. Bohn, University of Illinois

Matthew E. Hudson, University of Illinois

Glenn R. Johnson, University of Illinois

Frederic L. Kolb, University of Illinois

Mark A. Mikel, University of Illinois

Objectives and Accomplishments:

This project focuses on a multi-faceted and integrated approach to educating and training the next generation of plant breeders, directed at increased numbers as well as enhanced quality of education. The approach includes: conducting cutting edge research on a characteristic essential to substantially and sustainably increasing maize production in the 21st century, utilizing applications of genomics to germplasm improvement, creating tools for modern corn improvement and plant breeding education, expanding and enhancing the plant breeding curriculum, and providing a framework of support for gifted students recruited from basic biology and mathematics and from underrepresented groups to truly grow the number of graduate students pursuing plant breeding as a career option. The project builds on objectives and progress in the Illinois Plant Breeding Center, established in June 2008, to increase MS and PhD graduates in crop genetic improvement 3-fold (compared to 2007 levels) to 25 per year.

Research objectives focus on high corn yield under high planting density, which is essential for the scientific community to doubling yields in the next 25 years. The specific research objectives are:

1. *Evaluate different combinations of inbreds from various elite heterotic subgroups to identify hybrid combinations that can yield proportionately higher under intense plant densities.*
2. *Identify QTLs in key heterotic subgroups through map-based approaches and/or association mapping and screen candidate genes to investigate factors underlying the characteristic.*
3. *Validate results and devise methods to utilize information in hybrid improvement selection schemes.*

Toward research objectives, 32 hybrids (every combination of a set of 4 female and 8 male lines) representing a broad range of germplasm relevant to US commercial corn hybrids were grown at 5 population densities (25K, 32K, 40K, 47K and 54K plants per acre) in 3 environments with varying levels of moisture availability (from summer drought to irrigated) across 2010 and 2011. An incomplete block design was employed, with population densities nested within hybrid entries. Data were collected on traits important to source-sink relationship (e.g. yield, yield components, grain fill), photosynthetic capacity (e.g. leaf angle, leaf area), plant architecture (e.g. stem diameter, tassel morphology), hormonal balance (e.g. plant height), and stress tolerances (e.g. barrenness, anthesis-silking interval), among others. There were five hybrids that yielded above 175 bushel per acre at the highest population densities across

environments. The parents of these hybrids will be the focus of a deep dive to identify QTLs associated with factors underlying the capacity for high yield under high population density.

A design for a connected population for QTL mapping has been developed to maximize mapping resolution, which utilizes 3 of each of the superior female and male parents. RILs (doubled haploid lines or single-seed descent lines) from all possible crosses of the female lines and from all possible crosses of the male lines were increased in the greenhouse in Winter 2010 to prepare for testcross hybrid production in Summer 2011 to create the 'connected population' of >300 hybrids. Testcross nurseries have been harvested and seed counts are underway to determine the number of hybrids to be grown in Summers 2012 and 2013. Furthermore, RILs were grown to V3 stage in the greenhouse to collect leaf tissue for genotyping. DNA extraction is being done in a manner to ensure high quality DNA for producing genotype-by-sequence data for each RIL. Genotypes of the testcross hybrids will be inferred from parental genotypes.

The specific educational objectives of the project are:

4. *Establish capability in dihaploid (doubled haploid) production to enable hands-on instruction in a new industrial technology and develop teaching materials to demonstrate its use as a tool in modern corn breeding.*
5. *Develop 1 new plant breeding course and upgrade 2 current courses to expand and enhance the plant breeding curriculum.*
6. *Develop a new course to provide a background in agriculture to students who received their undergraduate degree in a non-agricultural area to support graduate recruitment from disciplines related to basic biology and mathematics.*
7. *Provide summer internships in University of Illinois breeding programs for undergraduates from the University of Puerto Rico in support of graduate recruitment from underrepresented groups.*

Toward education objectives, a protocol for dihaploid production in corn was developed based on a review of the literature involving successful methodologies used in corn and other cereals, and implemented. The protocol can utilize either colchicine or other more environmentally-friendly chromosome doubling agents. Process efficiency was enhanced by developing methods for early detection of doubled haploids to eliminate false positives and prevent wasted greenhouse resources. Teaching materials were created for use in the new course on Advanced Plant Breeding to debut in Spring 2012.

A new course to provide a background in agriculture to new graduate students who received previous degrees in non-agricultural areas (e.g. Biology, Math, Bioinformatics) debuted in fall 2010: CPSC 419 – Midwest Agricultural Practices. A 'crash course' in agronomy, the course features 8 lectures on various basics related to farm practices and the agricultural value chain taught by experts in each area. Students receive a high-level introduction to topics key to field testing and cultivar improvement as well as

resources to direct further study in these areas. To date, 22 students have enrolled and several others have audited at least one class/topic.

In addition, CPSC 466 *"Genomics for Plant Improvement",* a literature-based survey course in applied plant genomics taught by Dr. Stephen Moose, has been upgraded to a format effective for classroom delivery or across distances. Five self-contained modules were created and made available to enrolled students through the Illinois Compass software system, which included copies of journal articles, Power Point presentations that highlighted important concepts and practices for each topic area, self-paced exercises that allow students to gain experience with genomics data analysis, and homework assignments that assess competence with course content. The Illinois Compass system also allows for discussion of course topics, and was used to field questions from students about homework assignments outside of class. The updated course is in its second term of instruction and has benefited 53 students in total so far. Fall 2011 enrollment for CPSC 466 is 19: 16 graduate students and 3 undergraduates.

To update CSPC 558 "Quantitative Genetics and Plant Breeding" taught by Dr. Martin Bohn, an interactive MATHEMATICA-based notebook was developed to provide students with the opportunity to explore quantitative-genetic concepts and how these apply to the design of plant breeding programs. Eight chapters of the 'notebook' covering topics from Hardy-Weinberg's Law to the use of linear mixed models in phenotypic selection in plant breeding and variety testing are designed to manipulate and visualize mathematical code on which quantitative-genetic concepts rest. All materials provided are interactive and can be controlled by the student to meet his/hers learning style and needs. This class was taught in its new format in Fall 2011.

The internship program for undergraduates from the University of Puerto Rico was implemented in Summers 2010 and 2011. Each year, applicants were recruited and pre-screened at UPR by collaborator Dr. Linda Wessel-Beaver, and then interviewed by an Illinois Plant Breeding Center leader in January/February to select students for internship awards. Although the 2010 program offered 10 weeks, the length of the internships was shortened to 6 weeks in 2011 to fit the altered academic calendar at UPR. With this change, we were able to accommodate 4 interns in 2011 versus 2 in 2010. Interns rotated through 3-4 different breeding programs involving corn, broccoli, miscanthus, sorghum, and wheat from May through August, 2011 (10 weeks total). The interns participated in planting, tissue sampling, pollinating, and harvesting activities in field, lab, and greenhouse. This experience provided exposure to an array of crops, breeding objectives, work settings, and research teams. Interns experienced campus life at UIUC; student housing and bicycles for campus transportation were provided. An exit interview and with the interns and written evaluations highlighted strengths and is used to guide program improvements for the following year. Based on student feedback, we intend to stay with the 6 week format which also allows us to accommodate more students.

Broad Impacts:

Graduate education in plant breeding is a primary target for long-term impact, by increasing the number and diversity of plant breeding professionals well prepared to lead industrial and academic research programs and make vital contributions to crop improvement. Furthermore, increasing the

quality of this educational preparation will have lasting and broad-reaching impact, especially as graduate numbers from the University of Illinois increase. In particular, we look to increase the number of quality-trained plant breeders for the plentiful industrial positions based in Puerto Rico at winter/continuous nursery facilities.

Evidence of progress toward these targets is seen by

- An increase in the number of students in the Illinois Plant Breeding Center, from 21 in 2007 (and earlier) to 58 in 2011, a 2.76-fold increase.
- An increase in the caliber of student applicants to the Illinois Plant Breeding Center; Fall 2011 applicant pool had an average GPA of 3.7.
- Several past UPR interns have taken steps to pursue an educational pathway/career in plant breeding e.g. V. Olivera has begun an MS program in plant breeding at UPR, K. Navarro interned with Pioneer Hi-Bred International at their Ivesdale, IL corn breeding station in Summer 2011.

The internship program structure and success was leveraged to solicit support from Dow Foundation to expand internship opportunities for under-represented groups as well as military veterans (6 internships in total), enhancing our intern sponsorship, outreach, education and recruiting programs for future plant breeders.

Moreover, students have opportunity through research to explore a trait that is essential to doubling yields in the near future: high yield under high population density. Having one location under irrigation allows us to see the impact of moisture stress on intense interplant competition. Furthermore, because some inbreds contributed to both high-yielding and low-yielding hybrids, we have the opportunity to explore general and specific combining ability in a high plant density environment. We anticipate new insights into factors and interactions underlying high yield under intense plant-to-plant competition will come from this work.

The research conducted with this project has also had an educational impact. An undergraduate worker, Mallory Plocher, took a job with Bayer CropScience in their cotton breeding program at Memphis, TN after completing her BS in Crop Sciences. Others are investigating graduate opportunities in plant breeding.

Deliverables:

Publications:

Choe E., C. Hayot Carbonero, K. Mulvaney, A.L. Rayburn, and R.H. Mumm. 2011. Improving *in vivo* maize doubled haploid production efficiency through early detection of false positives. Submitted to Plant Breeding journal.

Oral/ Poster Presentations:

Mansfield, B.D., and Rita H. Mumm. 2011. Survey of plant density tolerance in US maize germplasm. Poster and abstract at ASTA Seed Expo, Chicago, IL, 7-9 December 2011.

Potts, S.M., and R.H. Mumm. 2011. Utilizing 'connected populations' for QTL discovery. Poster presentation and abstract in the Proc. Amer. Soc. Hort. Science 108th Annual Meeting, Waikoloa HI, 25-28 September 2011.

Mulvaney, K.A., E. Choe, and R.H. Mumm. 2011. Improving efficiencies in maize dihaploid production. Poster presentation at the Third Annual Research Symposium. Northeastern Illinois University Student Center for Science Engagement, Northeastern Illinois University, September 16, 2011.

Potts, S.M., B.D. Mansfield, and R.H. Mumm. 2011. Development of stress-tolerant maize lines under high plant density. Poster presentation at Agronomy Field Day, University of Illinois Farm, Urbana IL, 18 August 2011.

Potts, S.M., and R.H. Mumm. 2011. Utilizing 'connected populations' for QTL discovery. Poster presentation at NAPB/PBCC 6th Annual Workshop, College Station TX, 23-25 May 2011.

Choe, E., M.O. Bohn, R.H. Mumm. 2011. Establishing *in vivo* maize doubled haploid production at the University of Illinois for education and research purposes. Abstract and poster presentation at the Maize Genetics Conference,17-20 March 2011. St. Charles, IL. http://www.maizegdb.org/maize_meeting/2011/ MM2011.pdf

Community Resources Generated:

The research portion of this project has generated phenotypic data relevant to a set of ex-PVP lines for which seed is publicly available through GRIN. Likewise, this project is generating genotypic and phenotypic data relevant to RILs for which seed will be publicly available in the future. All serve as educational resources.

Teaching materials relevant to dihaploid production were created to facilitate instruction in this important area gaining momentum in plant breeding. Furthermore, USDA funding has supported the development of the structure and content for extension of plant breeding curricula to be delivered on-line; thus, impacts can reach beyond the UIUC campus. CPSC 466 was recorded with integrated audio and video of power point presentations and lectures archived, so that the course could be taken remotely and self-paced, without the need for any additional, specialized software.

Internship opportunities have been established and expanded for UPR undergraduates to experience plant breeding firsthand, to explore professional roles in crop improvement, and to sample the graduate school experience.

Training:

The training provided through this project vastly deepens and intensifies its reach and impact:

Stephen Phillips – MS-level technical manager assisting in seed and field aspects for all facets of the project; and Wendy White – MS-level coordinator for the Illinois Plant Breeding Center, organizing the UPR internships;

Eunsoo Choe and Christine Hayot Carbonero– post docs employed on the dihaploid project;

Brian Mansfield – MS candidate in the Mumm Lab researching population density effects;

Sarah Potts – PhD candidate in the Mumm Lab researching population density effects;

Christine Lucas – PhD candidate in the Moose Lab creating course materials.

Undergraduate intern, Kelly A. Mulvaney, from Northeastern Illinois University (supported on CREAR (Collaboration and Retention through Environmental and Agricultural Research) Project funded by USDA-NIFA HSI Grant No. ILLE-2010-02093) worked on the dihaploid project. Undergraduate student interns from UPR include Krystel Navarro, Gabriela Nazario Ramos, Virgilio Olivera, Sara Gonzalez, Pedro Cruz, and Veronica Brotons.

Undergraduate students involved in the high population density research include Joseph Brines, Sylwia Budzik, Kyle Carpenter, Daniel Hay, Brenda Ha, Kit Heller, Daniel Herriott, Haley Johnson, Danielle Lekas, Adam Massie, Lindsay Martinez, Sondra Monier, Russell Montgomery, Kord Nolte, Mallory Plocher, Emily Roberts, Bianca Rog, Jeffrey Trost.

New and updated plant breeding courses have already been relevant to 79 students so far, providing improved instruction to 59 graduate students and 20 undergraduates.

Collaborations:

Dr. Edward Buckler (USDA ARS, Cornell Univ.) and team will be involved in genotyping the connected population created to identify factors underlying high yield at high population density.

Dr. Nancy Wrinkle, Assoc.Prof. Mathematics, Northeastern Illinois University, Chicago, IL collaborated to facilitate and support undergraduate research internship of Kelly Mulvaney through CREAR (Collaboration and Retention through Environmental and Agricultural Research) Project funded by USDA-NIFA HSI Grant No. ILLE-2010-02093.

Dow Foundation Aid-To-Education Program, Katherine Armstrong, liaison.

Dr. Jeffrey Ross-Ibarra, Dept. of Plant Sciences and Genome Center, University of California at Davis is collaborating to share and extend use of genotypic data and phenotypic data collected on the ex-PVP diallel population.

Improving Drought Tolerance and Aflatoxin Resistance in Maize; Education, Extension, and Translational Breeding via Altered Lipid Metabolism

Seth C. Murray

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Tom Isakeit, Texas A&M University, t-isakeit@tamu.edu

Project website: http://aflatoxin.tamu.edu

Objectives and Accomplishments:

Research In this project period we tested 470 hybrids from an association panel that had been crossed to one of two isogenic versions of Tx714, one with knockout mutations for the *LOX4* gene (242) and one with knockout mutation in the *LOX5* gene (228). These mutants have been shown to condition quantitative variation in drought tolerance (*lox4*) and in aflatoxin resistance (*lox5*). Two water regimes, full season stress and well watered, were used along with inoculation of *Aspergillus flavus*. Yield and yield components (kernel row number, thousand seed weight, etc.), agronomic traits (height, flowering time, etc.), composition via near infrared spectroscopy, and aflatoxin content were measured. Significant differences were observed for the genotypes in aflatoxin accumulation and yield under drought. Summer and winter nurseries were grown to increase seed for additional hybrid testing in 2013 to be followed with association mapping.

As a separate objective of the study, *lox4* and *lox5* mutant alleles continued to be backcrossed into eight elite Texas adapted maize lines. Many of these lines are in the BC_3F_2 stage now and hybrids will be produced in the 2012 summer nursery for 2013 small plot and on-farm trials.

Education We continued to develop, refine and integrate our graduate level classes in Quantitative Genetics in Plant Breeding (Murray) and Molecular Plant Pathology (Kolomiets) to fit to a web based format for distance/ web-based education.

Extension We continued to develop and deliver extension programming to producers both through extension educational materials and presentations.

Broad Impacts:

Research: Both the *LOX4* and *LOX5* genes appear to have alleles with disrupted reading frames and others that are potentially absent. These may be used as a source of null alleles and easily selected in breeding programs for quantitative reduction in drought and aflatoxin stress. Multiple graduate students are being trained and producers educated.

Deliverables:

Publications: three additional publications are in preparation from project by students

Mayfield, K.L., S.C. Murray, W.L. Rooney, T. Isakeit, and G.A. Odvody. 2011. Confirmation of QTL reducing aflatoxin in maize testcrosses. Crop Science. 51:2489-2498.

Boote, K.J., A.M.H. Ibrahim, R. Lafitte, R. McCulley, C. Messina, S.C. Murray, J.E. Specht, S. Taylor, M.E. Westgate, K. Glasener, C.G. Bijl, and J.H. Giese. 2011. Position Statement on Crop Adaptation to Climate Change. Crop Science 51:2337–2343.

Isakeit, T., S. Murray, and J. Wilborn. 2011. Efficacy of Afla-Guard (*Aspergillus flavus* NRRL 21882) to control mycotoxins on corn in Burleson County, Texas, 2010. Plant Disease Management Reports 5:FC091. (refereed short technical report)

Isakeit, T., S. Murray, and K. Mayfield. 2011. Aflatoxin and fumonisin in transgenic corn hybrids in Burleson County, Texas, 2009. Plant Disease Management Reports 5:FC090. (refereed short technical report)

Oral/ Poster Presentations:

Barrero-Farfan, I. D., S.C. Murray, D. Pietsch, and S. Labar. 2011. Metanalysis of the Texas corn crop testing program. NAPB Annual Meeting; College Station, TX 5/13-25/2011

De La Fuente, G.N., I. Barrero-Farfan, S.C. Murray, M. Kolomiets, T. Isakeit, and Y.S. Park. 2011 Improving drought tolerance and aflatoxin resistance in maize via altered lipid metabolism. NAPB Annual Meeting; College Station, TX 5/13-25/2011

Hague S., E. Runge, S. Feagley, J. Aitkenhead-Peterson, C. Morgan, S. Murray, J. Foster and R.Vesey. 2011. Study abroad programs in the Department of Soil and Crop Sciences at Texas A&M University. ASA-CSSA-SSA Meeting. 10/16-19

Murray, S.C., M. Kolomiets, T. Isakeit, and G.D. De La Fuente 2011. Improving drought tolerance and aflatoxin resistance in maize; education, extension, and translational breeding via altered lipid metabolism. USDA awardees meeting 1/13/2011

Murray S.C., G. De La Fuente, T. Isakeit, M.V. Kolomiets, K. Mayfield, G. Odvody, Y-S Park, M.L. Warburton, J.C. Wilborn, W.P. Williams, and G.L. Windham. 2011. Techniques, technologies and approaches to improve maize aflatoxin resistance. Genetics of Maize Disease Workshop. Raleigh, NC. 2/20-23/2011.

Murray S.C., G. De La Fuente, T. Isakeit, M.V. Kolomiets, K. Mayfield, G. Odvody, Y-S Park, M.L. Warburton, J.C. Wilborn, W.P. Williams, and G.L. Windham. 2011. Improving pre-harvest aflatoxin resistance in maize: new genetic and phenotypic approaches NCC167 Corn Breeders Meeting. St. Charles, IL. 3/15-16.

Yan, Y.,Y.-S. Park, S. Christensen, E. Borrego, X. Gao, G. De la Fuente, K. Mayfield, S.C. Murray, H.Wilkinson, T. Isakeit, W.-B. Shim, R. Meeley, and M. Kolomiets. 2011. Modulating lipid-derived signaling to improve corn traits. NAPB Annual Meeting; College Station, TX 5/13-25/2011

Extension presentations:

"Aflatoxin in Corn" to over 500 growers at Bell County Expo, Belton, TX. Jan. 25th, 2011;

"Aflatoxin in Corn" to 40 growers at Row Crop Update Navarro County Expo Center. Jan. 28th;

"Aflatoxin in Corn" to over 120 growers at Blacklands Income Growth Expo. Feb 8th, 2011;

"Aflatoxin management in corn". 2011 Nueces County Crop Tour. Robstown, TX, Jul. 10, 2011. 40 attended.

"Aflatoxin". 2011 San Patricio County Field Crops Tour. Sinton, TX. June 9, 2011. 40 attended.

"Aflatoxin & commercial products Afla-Guard and AF-36". Hill County Crops Tour. Bynum, TX. June 15, 2011. 40 attended.

"Aflatoxin management". 2011 Colorado County Row Crop Tour. Nada, TX. June 22, 2011. 40 attended.

"Corn aflatoxin". 2011 Falls County Blackland Row Crops Tour. Westphalia, TX. June 20, 2011. 40 attended.

"Aflatoxin research". 48th Stiles Farm Foundation Field Day. Thrall, TX. June 21, 2011. 80 attended.

"2011 plant disease outlook, including aflatoxin". Fort Bend County Crops Tour, Rosenberg, TX. June 23, 2011. 25 attended.

"Afla-Guard timing trial. 2011 Ellis County Crop Tour". Avalon, TX. July 1, 2011. 40 attended.

"Aflatoxin management in corn". IPM Workshop – Texas Pest Management Association. San Marcos, TX. Feb. 1, 2011. 20 attended.

"Afla-Guard update". Upper Gulf Coast Feed Grain & Cotton Production Update. Bay City, TX. Jan. 11, 2011. 40 attended.

<u>Community Resources Generated:</u>

"Evaluation of atoxigenic strains of *Aspergillus flavus* for aflatoxin control in corn on commercial farms in Texas -2011" Extension publication

"Prevention of aflatoxin contamination of corn using AF-36 or Afla-Guard" Extension publication PLPA-FC009-2011

Sequences for *LOX 4* and *LOX 5* will be uploaded to NCBI after submission of the accompanying manuscript.

Training:

Ivan Barrero: PhD student – led aspects of hybrid seed production, and phenotyping

Gerald De La Fuente: MS student – led aspects of project yield trial, and sequencing

Yuanxin Yan: Postdoc –assisted with southern blotting

Assisted in achieving field and lab objectives in this period for this project:

Graduate students: Adam Mahan, Jim Wilborn; Undergraduate students: Ryan McHugh, Andrew Beamsley, Joseph Beard; High school students: Travis Rooney and David Rooney

TAMU, PLPA 613 (Advanced Plant Pathology). Laboratory on aflatoxin analysis of corn, November 8, 2011. Six plant pathology graduate students.

Collaborations:

Collaboration with CIMMYT in Mexico resulted in a test being grown last year. In part from this project, PI Murray was invited to participate in the Crop Science Climate Change Working Group, whose recommendations were published

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Partnership for Research & Education in Plant Breeding and Genetics at Purdue University

Herbert Ohm

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Project website: http://www.ag.purdue.edu/agry/genetics

Objectives and Accomplishments:

The initial primary objective was to educate/train 20 graduate students (MS/PhD) to prepare them for successful careers in Plant Breeding and Genetics. However, additional corporate/Purdue commitments for student support enabled the admittance into the program of additional students in fall semester 2011. There are a total of five MS students (two have completed their MS degrees) and 26 PhD students in the program. Fourteen faculty in five departments serve as major or co-major advisor for the 30 students in the program. There are corporate PhD staff on several of the student advisory committees; and the advisory committees are multidisciplinary, with disciplines represented as appropriate for the interests of the respective student and thesis research project. Thesis research projects focus on genetics and crop germplasm enhancement for abiotic environmental stress tolerance, improved nutrient uptake and/utilization, and biotic stress resistance. Students participate in classroom education as well as out-of-classroom learning activities, such as presenting posters and oral presentations at regional and national professional meetings as well as presenting thesis research plans and progress in the form of posters once each semester at the recently organized monthly Purdue Plant Science Socials, at which several plant breeding corporate representatives participate; and complete an internship activity in a corporate setting. Students also participate in Extension meetings/activities with crop and seed producers, interact with K-12 students in secondary school and Purdue campus settings, as requirements of a new course, YDAE591, developed to extend students' communications experiences beyond the classroom (Extension and K-12 activities). A number of undergraduate students in the BS Plant Genetics and Plant Breeding area of specialization, as academic advisees/research assistants in research programs of various faculty in Plant Genetics and Plant Breeding, interact with the various graduate students in the Plant Breeding and Education Partnership, and developing hands-on knowledge and experience in plant genetics and improvement research.

Broad Impacts/outcomes Students are communicating and experiencing educational activities in broad interdisciplinary contexts, and expanding participation at professional meetings as well as in local settings. These activities encourage students, and their advisors, to develop thesis research early in their degree studies. Interactions with corporate representatives at the Plant Science Socials and other settings provide opportunities to learn about a broad array of career opportunities and become acquainted with professionals in corporations and academia, and develop networking skills. Students are developing understanding of the general areas of plant genetics and crop improvement, and their specific area of research, in a broad context of corporate and academic settings and consider possible directions of their careers.

Deliverables:

Benjamin Campbell completed the MS degree, August 2011, and is studying for the PhD degree at the University of Minnesota.

Kristin Chandler completed the MS degree, May 2011, and is a Senior Research Associate at Pioneer Hi-Bred, a DuPont Company, stationed at LaSalle, CO.

Publications: Two manuscripts for journal publications will be submitted in December, 2011.

Campbell, BW, Y Liu Y Jin, K Wise, and H Ohm. 2012. Inheritance and mapping of stem rust resistance in wheat line PI 410966. Crop Science

Chandler, K, AE Lipka, H Li, B Owens, B Dilkes, E Buckler, T Rocheford, and MA Gore. 2012. The Genetic Architecture of Orange Endosperm Color in Maize. Crop Science.

Oral/ Poster Presentations: National/regional meetings - research posters (15), oral presentations (10); Extension activities (7); K-12 activities (8); internships (3).

Workshop Participation:

Sandeep Marla: Maize Ac/Ds Transposon Workshop, July 2011, Boyce Thompson Institute, Cornell University, Ithaca, NY.

Rima Thapa: 16th Summer Institute in Statistical Genetics, June, 2011, University of Washington, Seattle, WA.

Community Resources Generated:

Support from Industry partners in this grant (AgAlumni Seeds, AgReliant Genetics, ConAgraFoods, DOW Agrosciences, Indiana Crop Improvement Association, Pioneer Hi-Bred, United Soybean Board; and China Scholarship Council) allowed us to provide competitive research assistantships to attract the following 31 outstanding students: Benjamin Campbell, Trulie Campbell, Kristin Chandler, Ignacio Ciampitti, Ani Elias, Joshua Fitzgerald, Rachel Foley, Nicholas Labonte, Kin Lau, Amanda Leafgren, Raymond Lindsey, Melissa McDonald, Bemnet Mengesha, Jason Morales, Brenda Owens, Jieqing Ping, Mike Popelka, Alex Renaud, Martha Patricia Romero Luna, Marla Sandeep, Hannah Schneider, Jenae Skelton, Jin Sun, Rima Thapa, Kirsten Thomas, Tyler Tiede, Melissa Welch, Shaylyn Wiarda, Yanbing Xia, Xiangye Xiao, Siming Xu. NIFA funds in this partnership grant are used for partial thesis research S&E, travel for students to attend regional and national professional meetings, and travel for the external advisory council to meet at Lafayette, IN annually. Departmental funds provide some GRA support. College of Agriculture funds are committed to cover GRA support for one student in YDAE and one student in Extension, and Purdue University support provides fee remissions.

Collaborations: Given the interdisciplinary nature of students' thesis research, a number of active research collaborations among faculty in broad trans-disciplinary areas at Purdue have developed: plant genetics/breeding, plant physiology, plant biology, plant pathology, horticulture, agronomy, forestry, entomology, and extension/outreach educational contexts and K-12. Also, active collaborations with

corporate partners have developed: DOW AgroSciences, Pioneer Hi-Bred, KWS Seed Company UK; and M. Bohn, U. of Illinois; and R. Graf, Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta.

Genetics of Calcium Uptake by Potato Tuber

Jiwan P. Palta

Project Director: Jiwan P. Palta, University of Wisconsin, Department of Horticulture,

Madison, WI 53706 Email: jppalta@wisc.edu

Co-PDs: John Bamberg, Mike Havey and Shelly Jansky, University of Wisconsin – Madison; Terese Barta and Devinder Sandhu, University of Wisconsin - Stevens Point

Objectives and Accomplishments:

The goal of this project is to integrate research, education and extension in training future plant breeders. By understanding the genetics of tuber calcium uptake, we will improve tuber quality of cultivated potatoes. This will be achieved by the evaluation and enhancement of wild potato germplasm. Tuber internal quality is a major limiting factor for the US potato industry. Breeders invest time and money in producing advanced selections which, in the end, often fail because of tuber internal defects, tuber bruising or storage quality issues. Processors pay a bonus to the growers for better tuber quality and penalize growers for poor quality. Thus, there is a strong commercial incentive to produce tubers with good internal quality as well as a low incidence of bruising. Calcium deficiency is pervasive among fruit and tuber crops because calcium moves with water in the xylem and very little water moves to these organs. Cultivated potato (Solanum tuberosum L.) tubers generally have low tuber calcium levels. Many studies, including our own, have established that tuber quality can be improved by in-season calcium fertilization. An increase in tuber calcium has been demonstrated to reduce bruise susceptibility, storage rot and the incidence of internal defects such as internal brown spot and hollow heart. The specific objectives of the proposed study are to: (1) map calcium accumulating genes at the diploid level using a segregating F2 population derived from a cross between S. microdontum (mcd) and S. kurtzinium (ktz), representing the extremes of tuber calcium among wild potato species); (2) validate at the tetraploid level an association between calcium accumulation ability and enhanced tuber quality, (3) expose undergraduate biology students to research and career opportunities in plant breeding by teaching a short course in plant breeding (lab and lectures) at a four year campus at Stevens Point.

A linkage map using AFLP is being constructed using about 100 F2 progenies of diploid parents, (mcd and ktz). For this purpose we are using dominant AFLP markers as well as co-dominant SSR markers. In

parallel studies these progenies were grown in at two locations in greenhouses under controlled nutrient regimen to generate phenotypic data on the tuber calcium accumulation ability. Results from these studies show segregation for tuber calcium concentration.

In another study we have obtained over 40 transgenic clones of a chipping variety Atlantic overexpressing a vacuolar calcium transporter gene CAX1. This variety is known to have very low tuber calcium concentration and very poor internal quality. This variety was transformed with a plasmid containing the short version of the CAX1 gene driven by the 35S promoter, 35S::sCAX1, this construct was kindly provided by Dr. Kendall Hirschi. The objective of this study is to evaluate the effect of the increased transport of calcium into the vacuole in the tuber calcium content; and if this increased calcium in the vacuole could contribute to a better tuber quality. Results show that overexpression of CAX1 increases the demand of these plants for calcium for normal growth. These transgenic show deficiency symptoms of calcium under standard nutrition. We are now investigating the influence of this gene on tuber calcium uptake in relation to tuber defects. We are also attempting to determine if sequestering of calcium in the vacuole in the transgenic plants is resulting in calcium deficiency symptoms.

We are also making progress on the validation of the genetic association between tuber calcium and tuber quality using a tetraploid population. This population was derived from reciprocal crosses made between the cultivars Atlantic (low tuber calcium, poor tuber quality) and Superior (high tuber calcium, good tuber quality). About 300 clones from this cross were grown in a replicated field trial at the University of Wisconsin experimental station at Hancock, Wisconsin (located in the commercial potato production area of the state). Tubers were evaluated for tuber internal quality including the incidences of internal defects, soft rot and bruising. At the same time tubers were sampled for quantification of tuber calcium. Data from these evaluations suggest that there is a genetic association between tuber calcium and tuber quality.

As a part of the undergraduate teaching effort we offered a course *Molecular Biology: Plant Breeding to Combat World Hunger* (Biology 490) at the University of Wisconsin, Stevens Point from May 23-27, 2011. Fifteen students completed the course and received hands-on training in techniques in plant breeding and genetics. A wide range of information was covered during the course, including history of plant breeding, genetic resources, methods of pollination, seed and pollen viability, experimental design, analyses techniques, screening for disease resistance, hybrid seed production, marker assisted selection and genetically modified plants. Of the students registered for the class, four were involved in summer internships. These students also had opportunity to visit UW research station at Hancock.

Students received first hand field experience in conducting breeding trials.

Broad Impacts: Our teaching effort at the University of Wisconsin – Stevens Point could result in some undergraduate students pursuing careers in plant breeding. No effort is currently being made on systematically improving tuber internal quality and storage quality by genetic and breeding approaches. Our studies could potentially unveil new and novel means in this important area of plant breeding.

Deliverables:

Oral/Poster Presentations:

Zorilla, C., Palta, J.P., Navarro, F., Vega, S.E. and Bamberg, J. 2011. Breeding for improved tuber quality by interogressing tuber calcium uptake trait. Amer. Soc. Hort. Sci. Annual Meetings, Hawaii Sept 25-29.

Zorilla, C. and Palta, J.P. 2011. Over-expressing the vacuolar antiporter CAX1 in potato variety

'Atlantic': Phenotypic variations in the transformed clones. Potato Association of America Annual Meetings, Wilmington, NC August 14-18.

Other Products/Outcomes:

A number of students and scientists are getting training and experience on this project.

Training:

Undergraduate students – As mentioned above 7 undergraduate students received summer internships/training. Four additional students were involved in field trials and lab research.

Graduate students – Yong Suk Chung and Cinthya Zorrilla

Post-Doctoral/Assistant Scientists - Dr. Felix Navarro, Dr. Sandra Vega

Collaborations:

This project involves collaboration with three plant geneticists at University of Wisconsin - Madison, the USDA Potato Gene Bank at Sturgeon Bay, Wisconsin and two faculty members at the University of Wisconsin – Stevens Point.

Marker-Assisted Breeding to Enhance Disease Resistance in Corn, Rice, and Sugarcane

Prasanta K. Subudhi

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Objectives and Accomplishments:

Objective 1. Apply marker-assisted breeding to enhance resistance to *Aspergillus flavus* in corn.

The corn inbred lines, B73 (susceptible) and MP715 (resistant), were used for developing mapping populations to map the QTLs controlling resistance to aflatoxin accumulation. Two hundred sixty individuals each in F_2 and BC_1F_1 generation were selfed in 2011 growing season to generate F_3 and BC_1F_2 populations, respectively. For development of introgression lines, 120 BC_1F_1 plants were backcrossed to B73. Four hundred twelve simple sequence repeat (SSR) markers with whole genome coverage were screened for parental polymorphisms and 244 markers were polymorphic with a polymorphism rate of 59%. Both B73 and MP715 plants were inoculated with *Aspergillus flavus* strain 3357 using side needle technique and ears from both inoculated and uninoculated plants were collected for identification of differential expressed genes using cDNA suppression subtractive hybridization.

Objective 2. Develop molecular markers linked to bacterial panicle blight resistance in rice.

A recombinant inbred line (RIL) population is under development from the cross between the partially resistant variety Jupiter and the susceptible variety Trenasse for mapping the genes for resistance to bacterial panicle blight (BPB). In 2011 season, 300 F_4 plants derived from this cross were grown in the Rice Research Station and F_5 seeds were collected. Parental polymorphism survey using 900 SSR markers revealed only 207 markers indicating a high genetic similarity between parents. Additional SSR markers will be screened to obtain more polymorphic markers to ensure coverage of whole rice genome.

Objective 3. Develop molecular markers associated with leaf scald resistance in sugarcane.

Thirty three proteins associated with leaf scald resistance were identified from spots differentially expressed in response to infection by *Xanthomonas albilineans.* A population has been selected to study the genetics of leaf scald resistance. SYBR green and Taqman probe qPCR assays developed for the detection and quantification of *X. albilineans* (*Xa*) populations in infected plants were used to quantify bacteria in three resistant and three susceptible cultivars in the greenhouse and field. The populations detected by qPCR followed similar trends to disease severity ratings and vascular infection results. Low bacterial populations were found in newly emerged, systemically infected leaves of resistant cultivars. In a greenhouse experiment, 15 cultivars with a range of leaf scald susceptibility were inoculated with *X. albilineans* and the susceptible cultivars had higher populations of *Xa*. However, no bacteria were detected in five cultivars, four of which exhibit variable levels of susceptibility in field inoculation experiments. In a second experiment, plants of all cultivars showed symptoms in the inoculated leaves but did not develop systemic symptoms of infection.

Objectives and Accomplishments:

The project's educational and outreach objectives are: (a) to train graduate students in plant breeding, biotechnology, and plant pathology, (b) to develop a graduate level course on application of genomics for crop improvement, and (c) to develop and implement a summer training program to incite interest in plant breeding and biotechnology among the high school students.

Three graduate students have been recruited to undertake research projects in corn, rice, and sugarcane. A graduate level 3-credit course entitled 'Applied Plant Genomics' (AGRO 7080) has been approved by the College of Agriculture and Graduate School and is offered for the first time in fall 2011.

A total of five trainees (three students from underrepresented group and two biology teachers) from two different high schools participated in a two week-long 'Plant Breeding Summer Training Program' during July 11-22, 2011. The training program consisted of class room lectures, hands-on-training in laboratories, and field tours to acquaint the trainees of the potential of plant breeding and genetic tools in improvement of crops of agronomic and environmental importance. In addition to the PD and Co-PDs, several faculties and crop specialists from both on-campus and research stations were invited as guest lecturers.

Broad Impacts:

Molecular markers developed in this project will help in breeding disease resistant varieties in rice, corn, and sugarcane. The identification of proteins associated with the resistance response to *X.albilineans* infection in sugarcane will improve understanding of the molecular mechanisms of resistance. The project will provide an excellent opportunity to graduate students to acquire knowledge, expertise, and experience required to lead successful plant breeding programs in future. Exposure of the high school students particularly from the minority communities through the plant breeding summer training program will inspire them to choose career in plant breeding and genetics.

Deliverables:

<u>Oral/ Poster Presentations:</u>

Subudhi, P.K., J. Ham, C.A. Kimbeng, J.W. Hoy, and N. Baisakh. 2011. Marker-assisted breeding to enhance disease resistance in corn, rice, and sugarcane. USDA-NIFA Agriculture and Food Research Initiative, Plant Genome, Genetics, and Breeding Programs Project Directors Meeting, San Diego, CA, Jan 14, 2011. Annual Report, pp152-154.

Ham, J., H.S. Karki, B. Shrestha, I.K. Barphagha, R.A. Melanson, R. Chen, D.E. Groth, X. Sha, H. Utomo, P. Subudhi, and M.C. Rush. 2011. Molecular genetic and genomic studies on bacterial panicle blight of rice and its causative agent *Burkholderia glumae*. Phytopathology 101:S266.

Training:

Ramesh Dhakal: Graduate student in corn aflatoxin project.

Bishnu Shrestha: Graduate student in rice panicle blight project.

Andreas Fel Gutierrez Viveros: Graduate student in sugarcane leaf scald project.

Following high school students and teachers participated in 2011 Plant Breeding Summer training.

Ms. Shalanda Jordan (Biology teacher, Belaire High School).

Ms. Kandi Thompkins (Biology teacher, Tara High School).

William Veal and Austin Patrick Russel (High school students, Belaire High School).

Passion Kelly (High school student, Tara High School).

Collaborations:

Dr. Donald Groth, Rice Research Station, LSU Agricultural Center.

Dr. Xueyan Sha, Rice Research Station, LSU Agricultural Center.

Dr. W. Paul Williams, USDA Corn Host Plant Resistance Research Unit, Mississippi State.

Dr. Gary Windham, USDA Corn Host Plant Resistance Research Unit, Mississippi State.

Enhancing Leaf Spot Resistance in Peanut

Barry L. Tillman

Project Director: Barry L. Tillman, Agronomy Department, North Florida REC, University of Florida, btillman@ufl.edu.

Co-PDs : Maria Gallo, Agronomy Department, University of Florida mgm@ufl.edu;

Heather Kent, Florida Cooperative Extension Service, University of Florida hckent@ufl.edu.

Objectives and Accomplishments:

The goal of the proposed research is to provide tools and information for breeders to develop leaf spot resistant peanut cultivars. Specific objectives are: 1) Determine if antioxidant capacity and seed calcium content are related to germination and seedling emergence in breeding populations diverse for those traits, 2) devise inexpensive and rapid assays to test for those factors related to reduced germination, and 3) produce transgenic peanuts expressing *SAG12-IPT* and evaluate them for leaf spot resistance.

Work in 2010 showed that calcium concentration has a significant genotypic component. Broad-sense heritability was 33% in a group of peanut cultivars and experimental lines. Without prior knowledge of seed calcium concentration, genotypes were categorized based on market type (Virginia vs. Runner) and within the runner types, those with and without leaf-spot resistance. Seed of runner types without leaf spot resistance had the highest calcium concentration (1166 g kg^{-1}) followed by the Virginia types (1060 g kg^{-1}) and the leaf spot resistant runner types (955 g kg^{-1}). Work in 2011 found a positive correlation between seed calcium concentration and seedling emergence (r=0.22; p=0.0098) and a negative correlation between seed calcium concentration and leaf spot ratings (r=-.022; p=0.0087). Distribution of seed calcium concentration and leaf spot ratings appeared to be bimodal among several $F_{2:4}$ populations and seedling emergence appeared be to normally distributed. Several $F_{2:4}$ genotypes were identified which had good leaf spot resistance, high seed calcium concentration and very good seedling emergence.

The project will integrate education and research to address the long-term education goal: "to increase the number of students and scientists trained in plant breeding…". The fourth objective of this project is to develop an active learning environment via the 4-H Youth Development Program to educate high school teachers and students about the role of plant breeding in sustainable food production and create awareness of careers in plant breeding.

During 2011, a total of ten lesson plans were finalized (five lessons for middle school and five lessons for high school aged youth). The lesson titles are: 1) What is Plant Breeding?, 2) History of Plant Breeding, 3) Genetics of Plant Breeding, 4) Plant Breeding Ethics, and 5) Food for the Future. The lessons include both experiential and inquiry learning activities to introduce youth to the field of plant breeding and plant breeding careers. Lessons explore the history of plant breeding, basic plant genetics, and practical applications of plant breeding to solve problems. The content is aligned with the National Science Education Content Standards and 4-H Science Abilities. Piloting of the lessons, as well as development of a website to host the curriculum is underway.

Broad Impacts:

Despite over 30 years of breeding for resistance to leaf spot, control of the disease continues to rely on use of fungicides at considerable economic and potential environmental cost. A breakthrough in developing and deploying leaf spot resistant cultivars would create significant cost savings for farmers.

Development of a comprehensive curriculum to introduce middle and high school students to the science of plant breeding and related careers would serve the community for many years, and help promote the field of plant breeding as well as understanding of the important work that plant breeders do.

Deliverables:

<u>Publications:</u>

Burns, S., M. Gallo, B.L. Tillman. 2011 Expansion of a direct shoot organogenesis system in

peanut (Arachis hypogaea L.) to include US cultivars. In Vitro Cellular &

Developmental Biology – Plant. 1-9. Online.
http://www.springerlink.com/content/k63r84823467951p/

Oral/ Poster Presentations:

Thornton, S.T. 2011. Determining the relationship between field emergence and late leaf spot resistance in a breeding population of runner peanuts. Pp. 55 in Poster Abstracts of the National Association of Plant Breeders Annual Meeting. May 23-25, 2011. College Station, Texas.

Thornton, S.T., M. Gallo, and B.L. Tillman. 2011. Determining the Relationship between Field Emergence and Late Leaf Spot Resistance in Peanut. Pp. 39 in Proceedings of the American Peanut Research and Education Society Annual Meeting. July 12-14, 2011. San Antonio, TX.

Kent, H.C., J. Venn, B. Tillman, and M. Gallo. 2011. "Food for the Future": A New 4-H Plant Breeding Curriculum. Proceedings of the National Association of Extension 4-H Agents (NAE4-HA) Oct. 24, 2011.

Kent, H.C., J. Venn, B. Tillman, and M. Gallo. 2011. "Food for the Future": A New 4-H Plant Breeding Curriculum. Youth Development Institute, Gainesville, FL (January 24-26th, 2011).

Training:

Scott P. Burns, MS, 2010, Univ. of Florida; Transgenic and tissue culture research.

T. Steven Thornton, Ph.D. candidate, Univ. of Florida, 2009-present; Seed calcium concentration.

An Integrated Approach to Breeding Resistance to Phytophthora Capsici in Pepper

Allen Van Deynze

Project Director: Allen Van Deynze, University of California, Davis, avandeynze@ucdavis.edu

Co-PDs: Theresa Hill (tahill@ucdavis.edu), Raoul Adamchak , Hamid Ashrafi(Ashrafi@ucdavis.edu), University of California, Davis, James Prince (jamespr@csufresno.edu), California State University, Fresno, CA

Project website: sbc.ucdavis.edu and http://studentfarm.ucdavis.edu.

Objectives and Accomplishments:

Objective 1. Develop an interactive, hands-on education program for breeding and genetic diversity of peppers for undergraduates and K-12.

- Developed diversity panel of pepper for K-12 educational program and Pepper Diversity Garden visiting the UC Davis student farm
- Spring 2011, 705 children, 1st through 3rd grade received pepper plant breeding information in the form of written handouts, hands on pepper transplant activity, in-field demonstration and presentation. These children represented 12 schools in the Sacramento region.
- Thirty-five undergraduate and graduate field trip docents were trained in concepts of pepper breeding and how to teach these concepts to children. The pepper transplant activity was well received by field trip groups and docents.
- 50 High School students were given pepper plant breeding presentations during visits to the Student Farm.
- 62 Undergraduate students from both UC Davis and other colleges were given pepper plant breeding presentations at the Student Farm.
- Breeding pepper for disease resistance was presented to the California Seed Association student tour.
- Plant breeding information was presented by R. W. Adamchak in undergraduate Plant Science 49—Organic Production Practices lectures in Spring and Fall of 2011. The focus was on the possibilities of breeding for organic systems
- Ildi Carlisle-Cummins, a Community Development graduate student, was hired to replace Ethan Grundberg as the coordinator of educational part of the pepper project. Ildi has a strong background in education and grant funded projects (see resume below). Put in many hours to learn pepper plant breeding information, including visit and discussion with Susan Ashworth—seed saving author.
- Designed brochure for art and science fusion project here on campus related to pepper plant breeding.
- Worked with UC Davis Dining Services to plan for both seed saving events and integrating peppers and plant breeding educational materials into the UC Davis dining commons, as part of their sustainability program.
- Pepper Power Point on Seed Savings for Peppers completed and posted on project websites. Will be used as the basis for Seed Saving Workshops to be given in Winter Quarter 2012.
- In conjunction with the National Association of Plant Breeders and IssuesInk, (Seedworld magazine), organized interviews for 23 plant breeders and students at the National Association of Plant Breeders meeting in College Station, TX. Thus far 30 videos aimed at recruitment have been created and launched under a dedicated site for NAPB-Giant Views http://www.seedworld.com/. These pages have been cross-linked in several websites including eXtension.org and sbc.ucdavis.edu

Objective 2. Define and characterize the genetic basis of race-specific disease resistance to root rot and foliar blight on an ultra-high density genetic map of pepper

In 2011, we refined root rot and leaf testing protocols for disease screening as data were inconsistent among replicates. The following strategies have been tested:

- A detached leaf assay has been developed using macerated mycelium to test for foliar blight resistance.
- A whole-plug detached leaf assay is also being evaluated.

- A promising non-zoospore-based soil inoculation technique (using mycelium grown in liquid culture onto and into millet grains or vermiculite) is in the early stages of being introduced to this project to evaluate root rot resistance in any isolate that is not sporulating well.

To maintain virulent isolates, 20 new isolates have been collected from four different geographic locations in Central California. Four isolates have been screened on a subset of the RILs using the foliar blight technique. The full set of RILs will be screened with these isolates for foliar blight resistance. Three isolates are being screened using the zoospore inoculation technique for root rot resistance on the RILs.

Field performance was evaluated of the RIL population in 2 locations for horticultural and fruit quality traits and seed was increased for P. capsici testing experiments.

From 31,000 unigenes genotyped, we have resolved the genetic map into 897 unique genetic bins, representing over 3860 unigenes. The map was constructed with RECORD and distances calculated using JoinMap 4.0. This map has been compared (2632 common markers) to our primary map with 16,188 unigenes and 2886 genetic bins in a C. *frutescens x C. annuum* cross with 119 RILs (see https://pepchip.genomecenter.ucdavis.edu/) and will serve as the basis for QTL analyses.

Objective 3. Combine/pyramid genetic loci for *Phytophthora capsici* resistance in a hot pepper line using marker assisted breeding.
Field performance was evaluated of the RIL population in 2 locations for horticultural and fruit quality traits and seed was increased for P. capsici testing experiments. Based on data collected in disease screens, horticultural and quality traits, about 20 crosses were made combining lines with moderate to high resistance to P. capsici.

Broad Impacts:

The broad impacts achieved thus are in outreach, recruitment and training of high school, undergraduate and graduate students. Specifically,

1. Trained 90 non-plant science undergraduate students using experiential learning in the field on the importance of diversity in crop development, including crossing and see saving modules. Additional presentations were given to college students from Canada.
2. Exposed 705 1st-3rd grade students from 12 schools and 30 high school students on the importance of diversity and breeding thru experiential learning on the student farm.
3. Created online and hardcopy resources on pepper diversity and its use in breeding-accessed and module on seed saving.
4. Created 30 videos on plant breeding and student profiles.

Deliverables:

Publications:

Van Deynze, A Hill, T , Jim Prince, Yarne, S, , Rehrig, W. Reyes Chin-Wo, S. Ashrafi, H and Kozik, A. 2011. An Integrated Approach to Breeding Resistance to Phytophthora Capsici in Pepper. Plant and Animal Genome XIX, Jan 15-19, 2011.

Brewer H, Garcia C, Cheng D, Sidhu G, Bosland B, Van Deynze A, and Prince JP. 2011. A unified race scheme for Phytophthora capsici and the correlation of resistance in pepper with the presence of the Phyto 5.2 QTL. Proceedings, Plant and Animal Genome XIX.

Hill, T., A., H. Ashrafi, S. Reyes Chin-Wo, A. Kozik, and A. Van Deynze. 2010. Ultra high density EST-based maps reveal genome differences between C. frutescens and C. annuum. Plant and Animal Genome, San Diego, USA.

Oral/ Poster Presentations:

Van Deynze, Allen. June 23rd, 2010. Too much of a good thing: Challenges with high resolution genetic mapping. Plant Science Seminar, UC Davis. *30 Plant Scientists*

Shattuck, Jamie California Seed Association. Biotech Committee. Research at the Seed Biotechnology Center. September 14th, 2010-*30 plant scientists*.

Bradford, Kent, May 26th, 2010. Research at the Seed Biotechnology Center. Board of Directors, Nunhems Inc.- *10 Seed industry professionals.*

Van Deynze, A Hill, T , Jim Prince, Yarne, S, , Rehrig, W. Reyes Chin-Wo, S. Ashrafi, H and Kozik, A. 2011. An Integrated Approach to Breeding Resistance to Phytophthora Capsici in Pepper. Plant and Animal Genome XIX, Jan 14-18, 2011. Poster

Van Deynze, A. 2011, Chai Tai Group. University of California, Davis. Dec 2, 2010. *5 plant scientists from the largest vegetable company in Asia.*

Brewer H, Garcia C, Cheng D, Sidhu G, Bosland B, Van Deynze A, and Prince JP. 2011. A unified race scheme for *Phytophthora capsici* and the correlation of resistance in pepper with the presence of the *Phyto 5.2* QTL. Proceedings, Plant and Animal Genome XIX. Poster,

Hill, T., H. Ashrafi, S. Reyes- Chin-Wo, M. Solano Romero, A. Van Deynze, and A. Kozik. 2011. Comparisons of high-density EST-based maps in pepper species. Plant Biology, Minneapolis, MN. August 6-10, 2011. American Society of Plant Biologists.

Van Deynze, A., T. Hill, A., J. Prince, S. Yarnes, J. Chunthawodtiporn, W. Rehrig, S. Reyes Chin-Wo, H. Ashrafi, and A. Kozik. 2011. Development And Application Of Genomic Tools In Pepper. Plant and Animal Genome, San Diego, CA. Jan 15-19th, 2011.

Van Deynze, A., T. Hill, A., J. Prince, S. Yarnes, J. Chunthawodtiporn, W. Rehrig, S. Reyes Chin-Wo, H. Ashrafi, and A. Kozik. 2011. Development And Application Of Genomic Tools In Pepper. Solanaceae Disease Resistance workshop, Chiang Mai, Thailand. Feb 15-19th, 2011.

Van Deynze, Allen. Research at the UC Davis. Enza Zaden. San Juan Bautista, CA. Aug.16, 2011. Presentation

Van Deynze, Allen. Research at the UC Davis. HMClause. Davis, CA. July 7, 2011. Presentation

Community Resources Generated:

Publications have been in form of 5 videos co-produced with Vantage Inc. in Davis, CA, as well as contribution to outreach site on plant breeding and seed technologies. Videos can be seen at http://sbc.ucdavis.edu/publications/presentations_videos.html. Plant Breeding content can be seen at

http://www.plantsciences.ucdavis.edu/plantbreeding/index.htm, http://www.seedquest.com/keyword/seedbiotechnologies/default.htm. These include:

Bradford, KJ. 2010. Cross breeding lettuce. Ed. Whit Grebitus, Vantage Inc., Davis, CA.

Van Deynze, AE. 2010. Pepper breeding. Ed. Whit Grebitus, Vantage Inc, Davis, CA

Bliss F. 2010. Breeding rootstocks. Ed. Whit Grebitus, Vantage Inc, Davis, CA

Chetelat, R. 2010. Take a walk on the wild side. . Ed. Whit Grebitus, Vantage Inc, Davis, CA

Chan, S. 2010.Plants with one genetic parent. Ed. Whit Grebitus, Vantage Inc., Davis, CA

Plant Breeding websites include:

Van Deynze AE, Nelson, D. UC Davis Plant Breeding, Ed. Jeff Ross-Ibarra. http://www.plantsciences.ucdavis.edu/plantbreeding/index.htm

Van Deynze, AE, Nelson D. Bradford, KJ. 2010. Seed biotechnologies. Ed. Francois Korn. http://www.seedquest.com/keyword/seedbiotechnologies/default.htm The content on this website has been summarized and is posted on the PBGworks www.eXtension portal.

30 additional videos aimed at recruitment have been created and launched under a dedicated site for NAPB-Giant Views http://www.seedworld.com/.

Ethan Grundberg, Ildi Carlisle-Cummins, and R. W. Adamchak 2011. Seed saving modules for Pepper. http://studentfarm.ucdavis.edu.

Other products/ outcomes:

Workshops: Pepper and Persimmon Preservation workshop and Okra and Pepper Festival

Training:

William Z. Rehrig. PhD candidate on this project. William is managing the RIL population and proceeding with root rot screening of pepper. Field evaluation and disease screen development were performed by William. William has trained several students in genetic evaluation in pepper.

Jareerat Chunthawodtiporn. Jareerat is a Ph. D candidate who was trained in managing *P. capsici* cultures, inoculation and rating of pathogenicity in pepper at the Prince lab in Fresno and is working with William R. on this project.

Sebastian Reyes-ChinWo. Sebastian in a Research Assistant that was trained to generate high density maps using the RIL population for this project.

Marcelo Solano is a undergraduate intern from Costa Rica who was trained in analyzing high-density genetic maps and QTL analysis.

Paradee Thammapichai-undergraduate student trained in disease screens, greenhouse husbandry and seed processing in pepper.

Waqaar Arshad-undergraduate student trained in disease screens, greenhouse husbandry and seed processing in pepper.

Brittni Reid, an HS senior was trained in seed processing and field evaluation of pepper.

Shawn Yarnes. Shawn is a Postdoc who was trained to manage and evaluate pepper in the field as well as QTL mapping.

5 undergraduate students trained to pepper propagation and maintenance in the field for student farm.

Collaborations:

James Prince, pathologist at California State University Fresno.

Kurt Lamour, pathologist and leading expert on P. capsici

Roger Muren, Director of Research for Nunhems America

Paul Bosland. Pepper breeder, New Mexico State University.

Raoul AdamChak: University of California Davis student Farm Manager.

USDA-DOE Feedstock Genomics for Bioenergy Program Project Reports

(Alphabetical Order of Lead Project Director)

Functional Interactomics: Determining the Roles Played by Members of the Poplar Biomass Protein-Protein Interactome

Eric Beers

Project Director: Eric Beers, Virginia Tech, ebeers@vt.edu.

Co-PDs: Amy Brunner, Virginia Tech, abrunner@vt.edu; Allan Dickerman, Virginia Tech, dickerman@vt.edu; Richard Helm, Virginia Tech, helmrf@vt.edu

Project website: http://xylome.vbi.vt.edu/

Objectives and Accomplishments:

Our main objective is to apply our collection of over 350 wood-associated ORFs to the discovery of new protein-protein interactions that are important to wood formation. We are achieving this by using yeast two-hybrid (Y2H) assays to screen selected members of this wood-formation ORFeome against a cDNA library prepared from poplar xylem. Selected interacting proteins are subjected to more detailed analyses involving independent interaction assays and over-expression and/or silencing in transgenic poplar. To date we have completed or initiated 60 Y2H screens and we plan to screen 250 proteins. We are in the process of evaluating 11 transgenic poplar lines in field trials, and 10 new transgenic lines are being prepared.

Broad Impacts: Using proteins that are highly up-regulated in wood forming tissue as bait in Y2H library screens allows us to transcend the limitations of transcriptomics-based gene discovery projects by revealing interactome members that may not be differentially expressed in wood-forming tissues compared to other tissues. Our protein-protein interaction data are made public on our website following completion of each screen. In this way we hope to facilitate development of new testable hypotheses by members of the poplar research community and stimulate research on the regulation of woody biomass quality and quantity.

Deliverables:

Publications:

Petzold, H.E. M. Zhao and E.P. Beers, 2011, Expression and functions of proteases in vascular tissues. Physiol. Plant. published online October 24, 2011; doi: 10.1111/j.1399-3054.2011.01538.x

Rogers-Melnick, E., S.P. Mane, P. Dharmawardhana, G.T. Slavov, O.R. Crasta. S.H. Strauss, A.M. Brunner, and S.P. Difazio**, 2011, Contrasting patterns of evolution following whole genome versus tandem duplication events in *Populus*. Genome Res. published online October 5, 2011; doi:10.1101/gr.125146.111.**

Jia, X., M. Zhao, C. Zhao X. Sheng, A. Dickerman E. Beers, and A. Brunner, 2011, *Populus* biomass protein-protein interactions and their functions. BMC Proceedings 5 (Suppl 7):O38 ; doi:10.1186/1753-6561-5-S7-O38.

van Doorn, W.G., E.P. Beers, J.L. Dangl, et al. (19 authors, total), 2011, Morphological classification of plant cell deaths. Cell Death Differ. 18:1241-1246.

Zhao, C., A. Hanada, S. Yamaguchi, Y. Kamiya and E.P. Beers, 2011, The Arabidopsis Myb genes *MYR1* and *MYR2* are redundant negative regulators of flowering time under decreased light intensity. Plant J. 66:502-515.

Oral/ Poster Presentations:

Beers, E., Involvement of cysteine proteases XCP1 and XCP2 in tracheary element differentiation. 1st International Plant Protease Conference, Hemavan, Sweden, April 10-14, 2011.

Jia, X., M. Zhao, C. Zhao, X. Sheng, A. Dickerman, A. Brunner, and E. Beers, *Populus* biomass protein-protein interactions and their functions. Annual meeting, American Society of Plant Biologists, Minneapolis MN, Aug. 6-10, 2011.

Zhao, C. and E. Beers, Identification of potential posttranslational modifications of MYR2, a repressor of responses to decreased light intensity in Arabidopsis. Annual meeting, American Society of Plant Biologists, Minneapolis MN, Aug. 6-10, 2011.

Community Resources Generated: We have cloned more than 350 ORFs representing genes that are highly expressed in poplar wood-forming tissue. These ORFs are described at the project website and are available as gateway-compatible pENTR vectors on request. Many ORFs are also available as DB and AD fusions for Y2H assays.

Training:

Research Associate/Lab manager; *Xiaoyan Sheng

Lab Specialist Senior; *H. Earl Petzold

Research Scientist; *Chengsong Zhao

Postdoctoral Associate; *Ming Zhao

Graduate student; *Xiaoyan Jia

Agricultural Specialist; Deborah Bird

Undergraduate researchers; Stephanie Barker, Joe Edwards, Jim Meade, Sabina Page, Christine Romine, Nina Wilson

*Authors of publications or presentations listed above

Collaborations:

Matias Kirst, University of Florida; screening for proteins that interact with DUF579 proteins.

Laszlo Bako, Umea University, Sweden, and Ben Scheres, Utrech University, The Netherlands; detailed studies of interactions involving NAC domain proteins.

Center for Lignocellulose Structure and Formation (http://www.lignocellulose.org/); discovery of proteins that interact with glycoside hydrolases expressed in wood forming tissues.

Alfalfa Transcript Sequencing and SNP Discovery to Improve Biomass Composition

Charles Brummer

Project Director: E. Charles Brummer, The Samuel Roberts Noble Foundation, ecbrummer@noble.org

Co-PDs:

Maria Monteros, The Samuel Roberts Noble Foundation, mjmonteros @noble.org

Greg May, National Center for Genome Resources, gdm@ncgr.org

Objectives and Accomplishments:

Our goal was to increase the number of single nucleotide polymorphisms (SNP) available for alfalfa research and molecular breeding. SEQUENCING: We have sequenced transcriptomes derived from stems of 27 alfalfa genotypes (23 tetraploid and 4 diploid) using the Illumina Genome Analyzer IIx. De novo assembly of quality-filtered reads generated 25,183 contigs with a total length of 26.8 Mbp and an average length of 1065 bp, giving an average read depth of 55.9-fold for each genotype. We aligned the

contigs to *M. truncatula* annotated protein database using BLASTx. At E-value cutoff of 1e-10, 19,981 (79%) contigs hit known proteins in *M. truncatula* and matched 11,716 unique protein accessions. By realigning individual sequencing reads to the contigs, we detected 872,384 SNPs and 31,760 InDels under conditions of total uniquely aligned read no. > 20 and contingency test p-value <0.01. When 20 or more genotypes were required to each have at least 10 reads, 416,706 SNPs and 12,546 Indels were identified. We validated192 putative SNPs in 6 previously sequenced genotypes (4 tetraploid and 2 diploid) using high resolution melting (HRM). Of 179 SNPs with successful, specific amplification, 163 matched marker phenotypes for all 6 entries between sequence reads and HRM profile. The unmatched marker phenotypes are likely due to low read coverage and/or allelic specific expression. All sequence data is being prepared for public release through the Legume Information System (LIS) at NCGR. CANDIDATE GENE SNP: We identified 913 lignin biosynthesis and cell wall related genes from annotations in other species and 321 transcription factors that are either positively or negatively regulated during lignification in *M. truncatula*. We identified 33,543 SNPs within lignin and cell wall genes and 5,721 SNPs in 6 genotypes that are the parents of mapping populations. We have created a Legume Primer Database that allows query of SNP primers based on genomic location, polymorphism, or primer features, included GBrowser capabilities, and integrated them with the *M. truncatula* gene expression atlas (http://mtgea.noble.org/v2/). SNP MAPPING: Validated SNPs that were polymorphic between either of two sets of mapping parents contrasting for lignin content were further genotyped in a subset of progeny to identify SNPs with segregation ratios suitable for mapping in the corresponding mapping populations. Existing SSR-based genetic linkage maps were used as a framework to map the SNP markers relevant to lignin and cell wall development. To date, we have mapped 33 gene-associated SNPs relevant to lignin biosynthesis in alfalfa. QTL MAPPING: We have evaluated mapping population parents for lignin content and stem cell wall composition and have obtained or will obtain this coming year phenotypic characterization of cell wall composition in three mapping populations. Results of all these projects have been disseminated or will be disseminated through the scientific literature and invited and volunteered presentations.

Broad Impacts:

Our project has resulted in the first deep sequencing of the alfalfa transcriptome derived from stem tissue and the identification of thousands of potentially useful genetic markers for the alfalfa community. The project is enabling us to use the SNP we identified to develop an Illumina Infinium array of ~10,000 features which will be completed in the coming year. Subsequent analysis of array results will enable identification of a smaller number of SNP that produce unambiguous results which will be used to develop a GoldenGate array of 1526 SNP. Through this project we have developed collaborations with Forage Genetics and Pioneer to greatly expand the amount of marker data we can collect. These data will be publicly available. The SNP we identified are evenly distributed along the eight chromosomes of *M. truncatula*, a model species closely related to alfalfa, which will enable us to identify SNPs to use as markers for genome wide association and genomic selection applications. Population structure analysis for the 27 sequenced entries indicated that cultivated tetraploid alfalfa is clearly separated from subspecies falcata and wild diploid sativa, implying that those germplasms have potentially complementary favorable alleles useful for alfalfa improvement. Collectively, we have been able to

produce information that will be very useful for the development of genomic tools to accelerate alfalfa improvement.

Deliverables:

Publications:

Han, Y., Y. Kang, I. Torres-Jerez, F. Cheung, C.D. Town, P.X. Zhao, M.K. Udvardi, M.J. Monteros. 2011. Genome-wide SNP discovery in tetraploid alfalfa using 454 sequencing and high-resolution melting analysis. BMC Genomics 12:350.

Oral/ Poster Presentations:

1. Gou, J., Li, X., Han, Y., Khu, D.M., Brummer, E.C., M.J. Monteros. Development of SNP markers associated with biofuel traits in alfalfa. ASA-CSSA-SSSA International Annual Meetings. Oct. 16-19, 2011. San Antonio, TX.
2. Li, X., Y. Wei, A.D. Farmer, J.A. Crow, M. Wang, J. He, G.D. May, M.J. Monteros and E. C. Brummer. Single nucleotide polymorphism (SNP) discovery in Medicago sativa Using Illumina transcriptome sequencing. ASA-CSSA-SSSA International Annual Meetings. Oct. 16-19, 2011. San Antonio, TX.
3. Li, X., Wei, Y., Farmer, A., Crow, J.A., Wang, M., Ge, Y., May, G.D., He, J., Monteros, M.J., E.C. Brummer. Alfalfa transcript sequencing and SNP discovery to improve biomass composition. Plant Feedstock Genomics for Bioenergy (PFGB). Washington, D.C.

Community Resources Generated:

Transcriptomes from 27 alfalfa genotypes have been generated from stem tissue. These sequences are in the process of being deposited at GenBank and made available through the Legume Information System (LIS).

Training:

Postdocs – X. Li, J. Guo, Y. Han

Graduate students – A. Acharya

Other personnel – Y. Wei

A Systems Biology Approach to Elucidate Regulation of Root Development in Populus

Victor Busov

Project Director: Victor Busov, Michigan Technological University, vbusov@mtu.edu

Co-PDs:

Hairong Wei, Michigan Technological University, hairong@mtu.edu

Erik Lilleskov, USDA/Forest Service, elilleskov@fs.fed.us

David Weston, DOE/Oak Ridge National Laboratory, westondj@ornl.gov

Project website: http://treesbio.com/

Objectives and Accomplishments:

Objective 1) Generate systems-level knowledge and identify key regulators of root architecture in relation to N and water stress.

Objective 2) Identify genes that regulate root architecture under drought-prone and nitrogen-deficient environments via activation tagging.

During the period we have analyzed all expression data obtained from low nitrogen and drought treatments. Reconstruction of genetic networks was performed employing the ARACNE procedure. We have identified hierarchically-structured networks. We are in the process of producing transgenic plants with 14 key regulators in these networks. We have produced 3,500 lines and have screened 1,151 of them. Nearly 250 mutants (247) were discovered with modified responses to low nitrogen and drought. Of these 100 insertions were positioned in the genome and the putative tagged gene identified. A project site was established (http://treesbio.com/). We are working to present the data generated through the project in a searchable and most efficient format for the research community.

Broad Impacts:

Knowledge generated through this project will enable development of poplar varieties that can sustain robust biomass productivity under sub-optimal N and water environments.

Deliverables:

Publications:

1. Zawaski C, Kadmiel M, Pickens, J, Ma C, Strauss SH, and Busov VB (2011) Repression of gibberellin biosynthesis or signaling produces striking alterations in poplar growth, morphology, and flowering. Planta 234:1285-98

2. Gou, J., Ma C, Kadmiel M, Gai Y, Strauss SH, Jiang X, and Busov VB (2011) Tissue-specific expression of *Populus* C_{19} *GA 2-oxidases* differentially regulate above and below ground biomass growth through control of bioactive GA levels. New Phytologist 192: 626–639
3. Zawaski C, Kadmiel M, Ma C, Gai Y, Jiang X, Strauss SH, and Busov VB (2011) *SHORT INTERNODES*-like genes regulate shoot growth and xylem proliferation in *Populus*. New Phytologist. 191: 678–691
4. Xia Ye, Victor Busov, Nan Zhao, Rick Meilan, Lisa M. McDonnell, Heather D. Coleman, Shawn D. Mansfield, Feng Chen, Yi Li, and (Max) Zong-Ming Cheng. (2011). Transgenic *Populus* trees for forest products, bioenergy, and functional genomics. Critical Reviews in Plant Sciences. 30:5, 415-434
5. Yordanov, YS and Busov VB (2011). Boundary Genes in Regulation and Evolution of Secondary Growth. Plant Signaling and Behavior 6:5, 688-690
6. Yordanov Yordan, Sharon Regan and Victor Busov (2010) Members of the LATERAL ORGAN BOUNDARIES DOMAIN (LBD) Transcription Factors Family are Involved in Regulation of Secondary Growth in *Populus*. The Plant Cell. 22: 3662–3677

Oral/ Poster Presentations:

1. Victor Busov (invited) Regulation and evolution of growth patterns in a tree. Plant Science Center, Umea ,Sweden, May 2011
2. Victor Busov (invited) Division of labor: Multiple and specialized controls of vegetative growth and development in a poplar tree. June 2011, Porto Seguro, Brazil, IUFRO Tree Biotechnology Conference
3. Victor Busov A systems biology approach to elucidate regulation of root development in *Populus*. January 2010, Plant and Animal Genome Conference, San Diego, CA
4. Wilke, Aubree M, Burum, Justin, D, Smestad, Logan, Busov, Victor, B and Ralph , Steven. Genetic Control of Defenses Against Leaf-chewing Insects in *Populus*. August 2011, Minneapolis, MN, American Society of Plant Biology Conference

Community Resources Generated:

- Poplar activation tagging population of 3,500 lines. Contact PI for access or visit our website at http://treesbio.com/
- Searchable database for timetable of gene expression in response low nitrogen and drought. Visit our website http://treesbio.com/

Training:

Yordan Yordanov, Tatyana Georgieva, Hang Zang, Naomi Ojala, Elena Yordanova

Collaborations:

Dr. Steven Ralph: **NSF Plant Genome Award** 0922418 "**Genomic Approaches to Identify Insect Resistance Genes in Poplar Trees"**– We have provided approximately 1,000 lines for screening for resistance to defoliating insects.

Characterization of Nitrogen Use Efficiency in Sweet Sorghum

Ismail Dweikat

Project Director: Ismail Dweikat, Dept. of Agronomy and Horticulture, University of Nebraska, Lincoln, idweikat@unlnotes.unl.edu

CoPDs: Tom Clemente, Dept. of Agronomy and Horticulture, University of Nebraska, Lincoln, tclemente1@unl.edu

Don Weeks, Department of Biochemistry, University of Nebraska, Lincoln, dweeks1@unl.edu

Jianming Yu, Department of Agronomy, Kansas State University, Manhattan, jyu@k-state.edu

Objectives and Accomplishments:

The specific objective of this study is to incorporate improved nitrogen use characteristics into elite forage, sweet, and/or high biomass sorghum germplasm.

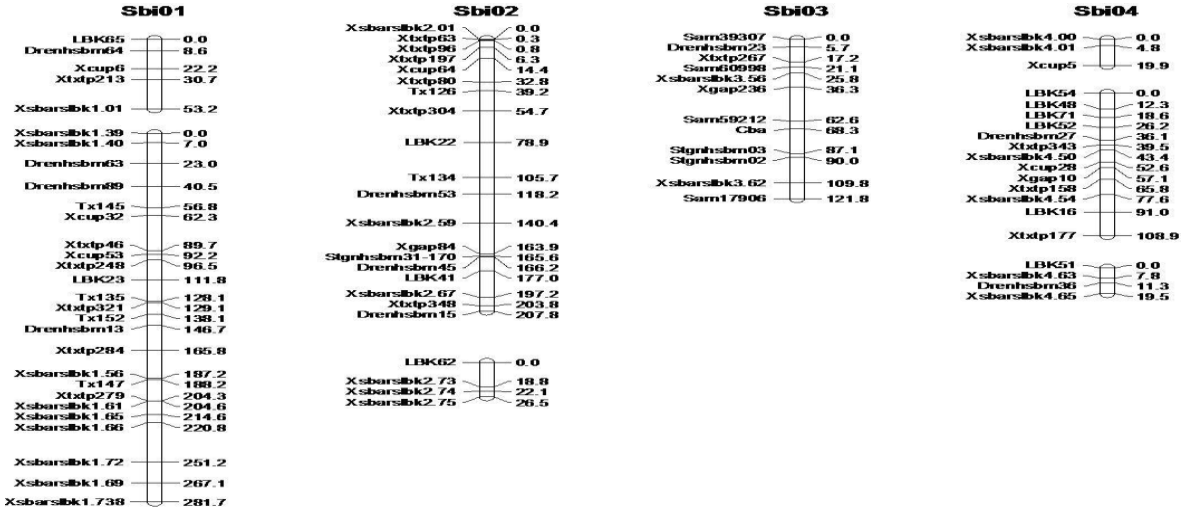
Introgression of genomic regions associated with improved nitrogen use into elite grain, forage sorghums and nitrogen efficient sorghum germplasm sources: In all cases the improved N-use QTL donor parents will be crossed to the selected grain, sweet and high biomass germplasm sources using standard plastic bag emasculation technique.

1. Crossed China 17 and San Chi San to several adapted forage and sweet sorghum varieties. The F_1 plants are being backcrossed to the recurrent parent (Sept. 2011).
2. We have screened 2200 SSR markers using DNA from the three parents (CK60, China 17, and San Chi San) with 40% polymorphism and mapped 146, and 154 SSR markers on Chiana17 X CK60 and San Chi San X CK 60 respectively.
3. Generated two genetic linkage maps of Ck60 X China 17 and CK60 X San Chi San.
4. We have collected chlorophyll data at three stages of development (8-leaf stage, flowering, and dough stage). The data will be incorporated into the genetic map. Additional data collected on the RILs were plant height and flowering date.
5. Total fresh and dry weight of above ground biomass and grain yield will be recorded this fall.
6. Genotyped by sequence the 215 RILs of CK60 X China 17 population. We expect to receive the data very soon. This activity was accomplished with Dr. Jianming Yu, Kansas State.

Ongoing Research:

1. Phenotyping the RIL lines of China 17 X CK 60 and San Chi San X CK60 and place the QTL associated with NUE traits on both created populations (in progress).

2. Collected phenotypic data will be incorporated to the linkage maps to establish association between markers (in progress).
3. Identify novel candidate genes for NUE using proteomic and gene expression profiling comparisons of high- and low- NUE RILs.
4. The effect of N regime on the proteome of the RIL parents (San Chi San, China 17 and Ck60) in the juvenile stages will be tested.
5. Determine the effect of N regime on the proteome of the RIL parental adult tissues.
6. We are conducting the genotyping–by-sequencing (GBS) through Restriction site Associated DNA (RAD) sequencing using the Illumina Genome Analyzer II platform. The RAD library will be generated with the CpNpG 5-methylcytosine sensitive enzyme, *SbfI*, which is predicted to yield approximately 15,000 unique hypomethylated *SbfI* sequences in an average sorghum genome. The RAD libraries of two parental inbreds (CK60 and SanChi San) will be first sequenced at about 40X coverage. The RAD libraries of the 192 RILs will be sequenced at about 6X coverage with bar-coding and multiplexing. Data processing and imputation will be conducted to generate a minimum of 2000 SNPs for this mapping population.



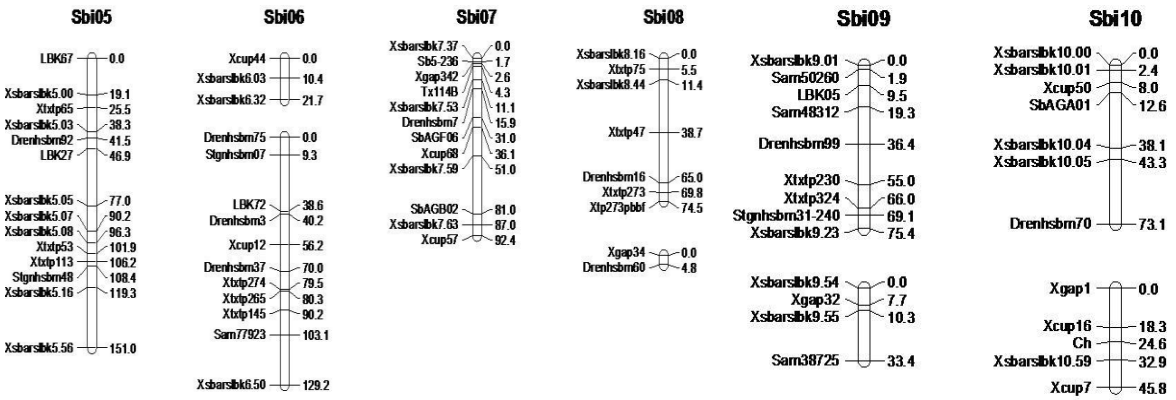


Figure 1. Genetic linkage map of sorghum based on 215 F6 RILs and 146 SSR markers of China17 X CK 60.

Genome-Wide Analysis of miRNA Targets in Brachypodium and Biomass Energy Crops

Pamela J. Green

Project Director: Pamela J. Green, University of Delaware, green@dbi.udel.edu

Objectives and Accomplishments:

The objectives of our project were to 1) Construct libraries for PARE (Parallel Analysis of RNA Ends) from different tissues and stress treatments of Brachypodium, and selected samples from Switchgrass, Miscanthus, and Sorghum; construct small RNA libraries from a subset of the samples. 2) Sequence these libraries to a depth of > 10 million reads and match to available genomic and cDNA sequence of the source species. 3) Identify and comparatively analyze miRNA targets and new small RNAs that cause target cleavage and evaluate their functional associations. 4) Identify tissue- and stress-regulated changes in target cleavage and other mRNA decay events. 5) Release the data to the public on a user-friendly website that provides comparative analysis tools.

To date, more than 80% of the proposed Brachypodium PARE libraries from tissue and stress treatments have been constructed. Ten libraries have been sequenced that generated a total of 676,164,268 raw and 589,600,052 genome-matched sequences. Additional libraries are either in the sequencing queue or submitted for quality control sequencing. An analysis of massive small RNA data from tissues and stress treatments resulted in the identification of over 100 novel, nonconserved annotated, and conserved miRNAs. These miRNAs were used to create a pipeline for miRNA target discovery from the PARE data.

Four PARE libraries have been analyzed with the pipeline, and PARE sequences were found for hundreds of target cleavage sites of the miRNAs identified under this project.

From switchgrass, several small RNA libraries and PARE libraries have been created and submitted for sequencing. Lacking a sequenced genome, a Bowtie library has been made from publicly available Switchgrass sequences, to which the small RNA and PARE sequences will be compared. The collection of plant material and analysis of RNA from additional switchgrass samples slated for library preparation is in progress. Tissue from Sorghum stress experiments has been received from our collaborator, and small RNA and PARE libraries are in the process of being constructed.

Broad Impacts:

In this study, we carried out an extensive analysis of miRNA targets identified by PARE sequencing. More than 20% of the target cleavage sites identified in Brachypodium tissue libraries matched the novel miRNAs that we discovered. Many of these miRNAs had low abundance levels that would be difficult to validate through other methods. In addition, tissue-preferential cleavages by distinct miRNA family members have been identified using the PARE data. Our PARE data are also useful for examining the cleavages and small RNAs that initiate the numerous phased small RNAs from Brachypodium that we previously reported (International Brachypodium Initiative, Nature 463:763-768,2010). This is evidenced by our initial analysis which revealed cleavages initiating phasing of both 21 and 24 nt phased siRNAs in the Brachypodium PARE data. The miRNAs that match the initiating cleavages include examples of well-known and new miRNAs. This indicates that PARE is a powerful tool to identify conserved and novel miRNAs that trigger phased siRNA production, in addition to its major application to miRNA target identification and cleavage validation.

Training: Skye Schmidt, Dong-Hoon Jeong, Linda Rymarquis, Sunhee Park, Monica Accerbi.

Collaborations:

Patricia Klein (Sorghum)

Todd Mockler & Jim Carrington (Brachypodium),

Switchgrass AP13 provided by Thomas Juenger

Long-term collaborator Blake Meyers

Organ and tissue-specific sucrose transporters: Important hubs in gene and metabolite networks regulating carbon use in wood-forming tissues of *Populus*

Scott A. Harding

Project Director: Scott A. Harding, University of Georgia, sharding@uga.edu

Co-PDs: CJ Tsai, University of Georgia, cjtsai@uga.edu

Objectives and Accomplishments:

Objective 1: Subcellular localization and substrate utilization of three phylogenetically distinct SUT proteins of *Populus.*

Subcellular localization experiments are being carried out using the *Agrobacterium rhizogenes* mediated hairy root system. To date, we have produced transgenic hairy root cultures from poplar leaf discs and stem sections for investigating GFP-tagged PtaSUT4. Experiments to characterize the effect of salicin, an abundant secondary metabolite highly characteristic of *Populus*, on PtaSUT function initially find that all three PtaSUT isoforms are sensitive to salicin and pH. Growth of yeast SUSY7/ura3 mutants was inhibited on sucrose media containing salicin but not on glucose media containing salicin.

Objective 2: Transgenic manipulation of sucrose transporter expression ratios in a tissue specific manner.

Preparation and testing of tissue specific promoters: Promoter sequences upstream of (1) a GDC subunit encoding gene expressed in leaf lamina in *Populus*; and (2) a tubulin encoding gene expressed in xylem in *Populus* were cloned and several promoter truncations were sub-cloned into GUS-based promoter analysis vectors, and successfully tested for tissue specificity and strength in tobacco and stably transformed *Populus* for their efficacy in the planned SUT manipulation experiments. Development of SUT Transformation constructs: We have generated synthetic chimeras for RNAi vector sub-cloning to manipulate xylem expression. For manipulation of SUT expression ratios primarily in leaves, we have constructed the necessary leaf-mesophyll-specific *PtaSUT4* silencing RNAi construct along with mesophyll-specific *PtaSUT3* and *PtaSUT5* over-expression constructs. Transformation of *Populus* is underway.

Objective 3: Manipulation of sink-source and water status of *SUT*-transgenics.

Soil water manipulation: Experiments to evaluate the effect of PtaSUT4 RNAi on poplar water use have been conducted using a range of soil water availability treatments. One very clear finding was that the rate of water uptake from soil that was allowed to dry was reduced in the transgenics compared to the wild type plants. Interestingly, biomass accrual by the transgenics was not reduced compared to the wild type plants despite the reduced water uptake, even when the treatment was repeated several times with the same plants over a period of several weeks. Comprehensive harvests and RNA isolation and delivery for RNA-seq have been completed. A manuscript describing a portion of the biomass and metabolite data tentatively entitled “Manipulating expression of the tonoplast-localized sucrose transporter in *Populus* (PtaSUT4) alters whole-plant water relations and photosynthesis” is in preparation.

Objective 4: Construction of correlation networks comprising genes and metabolites that impinge upon SUT.

RNA-Seq data analysis pipelines: Although transcript profiling was originally proposed to be conducted by microarray analysis using the redesigned Agilent microarray, we have now contracted for the less-

biased RNA-Seq approach as the cost has now become very competitive with the cost of microarray analysis. For this reason, we have established data analysis pipelines using the Bowtie-TopHat-Cufflinks tools suite.

Broad Impacts:
It is a little early as we are still developing the tools for many of the more innovative approaches planned. However, we are intrigued with the result we obtained from the acute water stress experiments with the existing SUT transformants which showed that shoot growth was not reduced even as water uptake was. This would seem to suggest some potential for learning something novel about the basic controls of water use efficiency in tree crops.

Deliverables:
Publications: In preparation: "Manipulating expression of the tonoplast-localized sucrose transporter in *Populus* (PtaSUT4) alters whole-plant water relations and photosynthesis"

Oral/ Poster Presentations:
Posters:
26th New Phytologist Symposium (Bioenergy Trees) Nancy, France, May 17-19, 2011: Sucrose transporter genes Populus: An investigation of their importance as regulators of biomass and carbon partitioning in trees. Harding SA, Frost, C, Tsai C-J
IUFRO Tree biotechnology 2011 'From genomes to integration and delivery', Bahai, Brazil June 26-July 2. Partial suppression of a strongly expressed tonoplast sucrose transporter affects water use and carbon partitioning in *Populus*. Harding, SA, Frost, CJ, Payyavula, RS, Tay, KHC, & Tsai, CJ.
University of Georgia Plant Center Retreat, Athens GA, Oct 27-28, 2011: Role of sucrose transporters in Populus resource partitioning and wood formation. Peng D, Harding SA, Tsai CJ.
University of Georgia Plant Center Retreat, Athens GA, Oct 27-28, 2011: A tonoplastic sucrose transporter plays a critical role in whole plant water relations in Populus. Frost C, Tsai CJ, Harding SA.

Oral:
Fifth International Poplar Symposium, Orvieto, Italy, September 19-23, 2010 The role of SUT4 in regulating carbon allocation, growth and phenylpropanoid homeostasis for plant defense in Populus. Harding SA, Payyavula R, Tay K, Tsai C-J.

Community Resources Generated:
Provisional and Utility Patent Applications related to the tonoplast SUT are on file.

Training:
Postdoctoral:
Chris Frost: Physiological characterization, RNA and metabolite extraction, lead author on publication in preparation entitled "Manipulating expression of the tonoplast-localized sucrose transporter in *Populus* (PtaSUT4) alters whole-plant water relations and photosynthesis".
Liangjiao Xue: Responsible for data network analysis; will soon lead RNA-seq analysis of samples from first drought stress experiments recently delivered for PE-50 Hi-seq.

PhD:
Duo Peng: Construction of SUT RNAi and over-expression vectors, cloning and testing of tissue-specific promoters to be used to drive the up and down-regulation of various combinations of SUT genes in tissue-specific manner.

ER65039, The Role of Small RNA in Biomass Deposition

Matthew Hudson

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Co-PDs:

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Steve Moose, University of Illinois, smoose@illinois.edu

Objectives and Accomplishments:

Objective 1: Identify active sRNA elements regulating cell wall deposition in feedstock grasses

To better characterize the role of sRNA in controlling biomass accumulation in the stems of feedstock grasses, we will sequence and profile sRNA, mRNA, and cmRNA from the middle stem, stem-apex, and stem-subapex at five stages of stem development from *Miscanthus X giganteus* (Mxg), switchgrass (*Panicum virgatum*), and prairie cordgrass (*Spartina pectinata*). The RNA sampling has been completed and this objective is at the sequencing and data analysis stage.

Objective 2: Elucidate the sRNA networks connecting flowering time, overwintering, senescence, and nutrient sequestration in perennial fuelstocks.

In Mxg, flowering is accompanied by an acceleration of rhizome development, interpreted as resulting from nutrient sequestration from senescing leaves and stems to the rhizomes. Our

preliminary data show that the Mxg rhizome and inflorescence are rich in sRNA, much of it previously uncharacterized. We also found that the down-regulation of an AP2-class transcription factor (ERF13) by miR172, which in maize regulates shoot maturation, is also present in Mxg. The shoot maturation pathway influences flowering time and other traits associated with perenniality. To better understand the role of sRNA in regulating perenniality-related processes, sRNA, mRNA and cmRNA will be sequenced and profiled from apical and reproductive tissues before and after flowering, and from overwintering vegetative tillers and rhizomes from Mxg, switchgrass, and prairie cordgrass. Again, this objective is at the data gathering and analysis stage.

Objective 3: Characterization of polyploidy- and hybridization-linked sRNA and their effect on gene expression in polyploid feedstock grasses

Polyploidy is often associated with increased growth, as well as genome-wide changes in gene expression, including sRNA. Mxg and many other highly productive C4 grasses (e.g.

sugarcane) are known polyploids. To gain new insights into the role of polyploidy- and hybridization driven sRNA on gene expression in polyploid fuelstocks, we are performing comparative developmental analysis of the sRNA and mRNA transcriptomes of leaves, stems, inflorescences and rhizomes between the allo-triploid Mxg and representatives of the parental species *M. sinensis* (diploid) and *M. sacchariflorus* (tetraploid). Sequencing is largely complete and data is being analyzed.

Objective 4: Evaluate the influence of sRNA on biomass composition and deposition using genetic transformation.

It is important to have methods for testing the effects of sRNA on the quantitative and qualitative biosynthesis of biomass, in particular genetic transformation of fuelstock species. To further this aim, an Agrobacterium-mediated transformation system was established for switchgrass. Within EBI, there are ongoing efforts to develop regeneration and transformation systems for Miscanthus species. Based on our preliminary data (Fig 3 and 4), our initial target for transformation is to study the effect of overexpression of mxg-miR397 on the biomass biosynthesis. This objective is currently at the planning stage.

Broad Impacts:

This proposal will bring together the previously disparate areas of biofuel crops, the analysis of small RNA populations and transcriptional profiling and bioinformatics. It will thus provide an example of a strongly interdisciplinary research project that has the potential to improve the breadth of education for a number of students. The data generated will provide useful examples for PI Hudson's graduate level bioinformatics programming class, co-PI Moose's undergraduate course in "Biotechnology in Agriculture", and a new graduate course taught by Moose on "Genomics for Crop Improvement". We have also budgeted for a significant component of paid undergraduate research, which will include one paid summer intern for each summer spanned by the project. Both PIs have a history of recruiting such interns from local community colleges. We anticipate that interns will participate in a broad range of activities, including molecular biology, field work, greenhouse plant culture and bioinformatics.

Deliverables:

<u>Oral/ Poster Presentations:</u>

"Discovering the sRNA-mRNA Regulatory Networks In Perennial Grasses for Bioenergy", ASA, CSSA and SSSA Annual Meeting (San Antonio, TX - Oct. 16-19, 2011), Symposium--RNA Profiling Applications to Crop Improvement

"Discovering the sRNA-mRNA Regulatory Networks In Perennial Grasses for Bioenergy", 6th Frontiers in Bioenergy, Purdue University, May 15th to May 81th, 2011.

<u>Community Resources Generated:</u>

We are still in the process of generating what should be the largest and most comprehensive dataset of small RNA, RNA and degradome data from a biofuel crop. This data will be made available to the community through repositories and our own website. Both of the undergraduates contributed to this dataset. Patrick Yoo made several Illumina libraries as well as performing RNA preps. Daniel Weber is focused on computational analysis of the very large datasets currently being generated.

Training:

Graduate: Daniel Weber

Undergraduate: Jin Ji, Patrick Yoo, Jiameng (Solaris) Wang

Mechanism of Carbon Partitioning Regulation by cpg13 in the Bioenergy Woody Crop Poplar

Matias Kirst

Project Director: Matias Kirst, Genetics Institute, School of Forest Resources & Conservation, University of Florida (mkirst@ufl.edu)

Co-PDs: Gary F. Peter, Genetics Institute, School of Forest Resources & Conservation, University of Florida (gfpeter@ufl.edu).

Project website: www.sfrc.ufl.edu/forestgenomics/

Objectives and Accomplishments:

Lignin is considered the main obstacle for the efficient bioconversion of wood cellulosics to renewable fuels, and its content is directly and inversely proportional to fermentable sugar yield. Lignin content is also highly negatively correlated with biomass productivity in several woody species. We previously identified a gene containing a DUF579 domain, referred hereafter as cpg13 (carbon partitioning and growth in LG13) as a key regulator of carbon partitioning to lignin and cellulose, and whole plant biomass productivity in a segregating Populus hybrid population. The **objectives** of this project are to: (1) characterize cpg13 function in the regulation of gene expression, metabolites, and cell wall chemistry and structure, and (2) determine the spatial and temporal expression and subcellular localization of cpg13 to advance our understanding of its molecular role. To modify the expression of cpg13 we developed transgenic lines using RNAi mediated gene silencing and ectopic expression to promote loss-

and gain-of-function, respectively. The coding sequence of cpg13, obtained from *P. trichocarpa* reference genotype Nisqually-1, was cloned into pCAPT Transitive for the RNAi and pCAPO for overexpression. Agrobacterium (strain GUV3101) mediated transformation was performed in the clone 717 (*P. tremuloides*) and 39 RNAi and 22 overexpression independent transgenic lines were regenerated. Regenerated lines were clonally replicated (3 ramets per genotype, 183 plants in total) and planted in a greenhouse at the University of Florida (Summer '10). Plants were grown in ebb-and-flow flood benches and experimental conditions were designed to maintain ideal conditions of light, temperature (16h Light:8h Dark, 28C) and nutrient for continuous growth (5mM for 6 weeks and 25mM for 4 weeks) throughout the experiment, until harvest. After 10 weeks the plants were harvested, and the organs (leaves, sylleptic branches, stem and roots) were dissected, dried and weighted. During collection xylem (i.e. entire stem below the bark) was harvested from each genotype for gene expression quantification. RNA from transgenic plants was extracted (Chang et al. 1993), double-stranded cDNA was synthesized and quantitative real-time PCR (RT-PCR) for transgene expression was performed. Lines with highest transgene effect (8 Overexpression and 9 RNAi) were selected for further analysis. These lines were clonally replicated and grown under the same conditions as before in 3 independent experiments that included wild type plants. Biomass and growth has been quantified for a minimum of 21 replicates per line, and data analysis is currently in progress. Xylem RNA was also extracted from these lines, to be used for microarray analysis, using in situ synthesized oligonucleotide microarrays containing one 60-mer probe for 45,555 predicted genes from the poplar genome. In parallel, the relative contents and profile of cellulose and lignin is being determined by Klason Analysis and pyrolysis Gas chromatography–mass spectrometry (pyGC-MS) using extractive-free xylem biomass.

Cpg13 protein localization was analyzed with 35s::cpg13:GFP (vector pEARLY-gate) construct stable transformed into Populus, and shows co-localization with lignin UV-Auto-Fluorescence in poplar petioles. Cpg13:GFP expression under cpg13 native promoter -2kb upstream- using vector pWGB4 is currently being transformed. In parallel, biochemical function analysis of the cpg13 protein is being performed. Cpg13 coding sequence without the predicted N-terminal signal peptide and transmembrane domain (first 34aa) was cloned into expression vector (modified pET15b - Novagen) and transformed in BL21(DE3) expression *E. coli* (Invitrogen). Bacteria were grown in Luria Bertani Medium with 1 M Sorbitol and protein expression was induced with 0.4mM of IPTG. The recombinant histag-cpg13 expression was confirmed by Western blot using anti-his antibody. The recombinant protein was purified with nickel affinity chromatography and 10mM of the 34kDa protein was obtained and confirmed by Liquid chromatography–mass spectrometry (LC-MS). Half of the total protein had his-tag cleaved using TEV protease. Finally, enzymatic activity assays are being performed to determine the biochemical function of the protein. Initial results from these assays suggest that the protein has methyltransferase activity.

Broad Impacts:

Improving our fundamental knowledge of the molecular basis for correlated growth and carbon partitioning will greatly enhance our ability to simultaneously improve biomass productivity while creating a more favorable chemical composition for bioconversion to biofuel. Understanding the role of cpg13 will support that objective. Furthermore, proteins with a DUF579 domain (such as cpg13) are

being increasingly associated with cell wall formation, but there is limited understanding of their biological function. The elucidation of that function in our project is, consequently, expected to have broader implications for the development of feedstock that is more suitable for bioenergy production.

Deliverables:

Oral/ Poster Presentations:

Ribeiro, C. L.; Novaes, E.; Dervinis, C.; Kirst. M. (2011) Functional characterization of a candidate gene for carbon partitioning in Populus,. Florida Genetics, Gainesville, FL.

Ribeiro, C. L.; Novaes, E.; Dervinis, C.; Kirst. M. (2011) Regulation of biomass growth and carbon partitioning in poplar - molecular characterization of a candidate gene. IUFRO Forest Tree Biotechnology Workshop, Arraial D'Ajuda - Brazil.

Ribeiro, C. L.; Novaes, E.; Dervinis, C.; Kirst. M. (2011) Functional analysis of a candidate gene involved in the regulation of biomass growth and carbon partitioning in populus. Plant and Animal Genome XIX Conference, San Diego, CA.

Training:

A Ph.D. level student is being currently funded through this project. Additionally, six undergraduate students have participated in the project, supporting activities such as tissue culture, and biomass and lignin quantification.

Collaborations:

Collaborations to support the characterization of wood chemical composition and enzymatic activity of cpg13 have been established with Dr. Wilfred Vermerris and Dr. Claudio Gonzales (both at University of Florida).

An Integrated Approach to Improving Plant Biomass Production

Jan E. Leach

Project Director: Jan E. Leach, Colorado State University, Jan.Leach@colostate.edu

Co-PDs: John McKay, CSU, John.McKay@colostate.edu; Daniel Bush, CSU, DBush@colostate.edu; Hei Leung, International Rice Research Institute, H.Leung@cgiar.org; Bingyu Zhao, Virginia Tech U, (bzhao07@vt.edu); Andrew Kern, Rutgers U kern@Biology.Rutgers.edu

Objectives and Accomplishments: Project initiated 10/2011
(1) Identify and validate genes that increase plant biomass accumulation in rice, using an integrated, systems biology approach to understand cause and effect.
We identified and characterized a mutant with increased biomass (2.2X) and increased seed yield (1.7X); gene discovery is in progress. A second IR64 mutant (G17490-1-B) with higher straw weight, more seeds and more tillers than the wild type was identified. A recombinant inbred population (1700 RIL) for two rice cultivars of biomass interest (IR64 X Aswina) was developed. A large scale phenotyping/genotyping of this population will occur in spring 2012. For genotyping by mapping, we completed an IR64 draft genome (70X) and the Aswina genome is in progress. Finally, eight rice biomass gene candidates are being validated by gene silencing/overexpression.

(2) Discover biomass Quantitative Trait Loci (QTL) in switchgrass.
We identified 14 F_1 plants derived from a cross between ca 'Alamo' and ca 'Dacotah'. These plants differ in biomass yield, flowering time, plant architecture, and disease resistance. Selected F_1 plants to generate seven pseudo-F_2 populations, which will be planted and evaluated in both greenhouse and field at both Virginia Tech and CSU.

(3) Validate/translate the role of candidate biomass genes in switchgrass.
We generated a construct for overexpression of the At*SHN*1 gene in switchgrass. A previous report suggested that the overexpression of At*SHN1* in rice could increase cellulose but decrease the lignin content in the transgenic rice plants. The At*SHN*1 construct is being transformed into switchgrass.

(4) Create a web based genomics platform display and analysis of rice and switchgrass comparative functional and genomic data. (None yet)

Broad Impacts: In addition to fundamental discoveries about biomass genes, we will greatly expand the genomic and genetic tool box for switchgrass. We will identify novel loci that are key contributors to biomass accumulation, cell wall composition and allocation in grasses.

Deliverables:
Publications (Including those from previous funding):

Jahn CE, JK McKay, R Mauleon, J Stephens, KL McNally, DR Bush, H Leung, JE Leach. 2011. Genetic variation in biomass traits among 20 diverse rice varieties. *Plant Physiol*155: 157-168.

Ainsworth EA and Bush DR. 2011. Carbohydrate export from the leaf - A highly regulated process and target to enhance photosynthesis and productivity. *Plant Physiol* 155: 64-69.

Feuillet, C, JE Leach, J Rogers, PS Schnable, K Eversole. 2011. Crop genome sequencing: lessons and rationales. Trends Plant Sci 16:77-88. doi:10.1016/j.tplants.2010.10.005.

Oral/ Poster Presentations:

Jahn, CE. 2011. Targets to Improve Quantity and Quality Lignocellulosic Feedstocks. Sustainable Bioenergy Development Center Seminar Series, Fort Collins, CO. November 29.

Tanger, P, C Jahn, E Wolfrum, N Santoro, M Baraoidan, H Leung, R Mauleon, K McNally, J McKay, D Bush, J Leach. 2011. Development of high-throughput phenotyping methods to investigate cell wall composition. Poster: International Rice Functional Genomics meetings, Taipei, Taiwan. Nov 7-9.

Leach, JE. 2011. Identifying Genes and Networks for Increasing Biomass Production in New Energy Grasses by Using Rice as a Model System. Symposium speaker, DOE/USDA-NIFA PI meeting, Crystal City, MD. April 20.

Jahn CE, L Derose-Wilson, J McKay, D Bush, H Leung, J Leach. 2011. Night-time stomatal conductance and transpiration negatively impact biomass accumulation. USDA-DOE Plant Feedstock Genomics for Bioenergy Awardes Meeting. Arlington, VA. April 10-13.

Broeckling B, M Baroidan, CE Jahn, J McKay, JE Leach, D Bush, H Leung. 2011. Identification of candidate genes using rice mutants for biomass engineering in switchgrass. USDA-DOE Plant Feedstock Genomics for Bioenergy Awardes Meeting. Arlington, VA. April 10-13.

Bordeos A, CE Jahn, JK McKay, JE Leach, DR Bush, H Leung. 2011. Biomass accumulation in wide crosses between wild and domesticated rice. USDA-DOE Plant Feedstock Genomics for Bioenergy Awardees Meeting. Arlington, VA. April 10-13.

Jahn, CE 2011. Genetic Analysis of *Oryza sativa*: Morphological and Physiological Traits that Contribute to Plant Biomass. CSU, Depart Soils & Crops seminar series, Ft Collins, CO. February 17.

Jahn, CE 2011. Using Rice as a Model to Identify Genetic Variation in Traits Related to Biomass. CO-WY USDA-ARS spring seminar series. Fort Collins, CO. February 14.

Bush, DR. 2010. Invited lecturer: The Biochemistry of Biofuels in Brazil, sponsored by The American & Brazilian Societies for Biochemistry and Molecular Biology (IUBMB, ASBMB, and SBBq). October

Jahn, CE 2010. Understanding Physiological and Morphological Traits that Contribute to Plant Biomass: Lessons from Rice. U Northern CO, Biology Seminar Series. Greeley, CO. October 29.

Jahn, CE 2010. Unraveling Plant Biomass Traits--Lessons from Rice. Western Great Plains Sustainable Feedstock Conference. Fort Collins, CO. September 15.

Leach, JE, CE Jahn, A Bordeos, M Baroidan, J Stephens, E Peachey, D Bush, H Leung, JK Mckay. 2010. Genetic Variation In Biomass Traits Among 20 Diverse Rice Varieties. Plant and Animal Genome XVIII. SanDiego, CA January 9-13.

Jahn, CE, I Ona, J Stephens, C Vera Cruz, D Bush, H Leung, J McKay, JE Leach. 2010. Screening a diverse set of rice varieties for variation in biomass and resistance to plant disease. Poster presentation at the 10th Japan-US Seminar: Genome-Enabled Integration of Research in Plant Pathogen Systems. January 24-28, Corvallis, OR.

Jahn, CE, J Stephens, B Mason, S Broadstone, DR Bush, H Leung, JK Mckay, JE Leach. 2009. Genetic variability and heritability of biomass traits in 20 diverse rice varieties. Poster P9-42, 6th International Rice Genetics Symposium, Manila, Philippines, Nov 16-19.

Jahn, CE. 2009 Approaches to Understanding Biomass Traits in Rice. Yunnan Academy of Agricultural Sciences, Kunming, China. August 10.

Leach, JE, CE Jahn. 2009. Crop Plants for Biofuels: Challenges and Opportunities. Lecture for Colorado Center for Biorefining and Biofuels: Professional Shortcourse. Ft Collins, CO. May 11-13

Community Resources Generated; Large recombinant inbred population of rice segregating for biomass traits (>1700 RIL); Mapping population of switchgrass.

Training:
Paul Tanger, PhD student, variation in cell wall composition; **Courtney Jahn**, PostDoctoral Fellow, physiological and morphological variation & gene discovery in rice; **Bettina Broeckling**, PostDoctoral Fellow, mutant analysis; gene discovery; **Amanda Broz**, PostDoctoral Fellow, gene validation in rice; **Leah DeRose-Wilson**, PostDoctoral Fellow, biomass QTL analysis; **Rene Corral**, Undergraduate, phenotyping; **Sasha Broadstone** (Undergraduate), phenotyping; **Jacqueline Johnson** (Undergraduate), phenotyping

Collaborations:
Rod Wing, Arizona Genomics Institute: genotyping of IR64 X Aswina RIL; **Stephen Klassen**, International Rice Research Institute: Large scale phenotyping

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Development of a Low Input and Sustainable Switchgrass Feedstock Production System Utilizing Beneficial Bacterial Endophytes

Chuansheng Mei

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Project website: http://www.ialr.org/images/stories/research/isrr/meiprojectreport9-28-11.pdf

Objectives and Accomplishments:

Objectives: 1) Global gene expression profiling induced by switchgrass bacterization with the beneficial endophyte *Burkholderia phytofirmans* strain PsJN (year 1); 2) Identify key genes from global gene expression profiling and study their functions (years 1-3); and 3) Analyze gross cell wall composition and saccharification efficiency after bacterial endophyte inoculation to monitor any potential structural and extractability changes as well as assess photosynthesis rate, water use efficiency, drought tolerance, and carbon sequestration (years 1-3).

Accomplishments to date: The project objectives have been successfully met. Using the DOE-funded switchgrass EST microarray, in a collaboration with the Genomics Core Facility at the Noble Foundation, we have determined gene expression profile changes in switchgrass induced by bacterization with the beneficial endophyte *Burkholderia phytofirmans* strain PsJN . Based on the analysis of the microarray data, we identified over 50 candidate genes for further qPCR verification. So far, five key genes (representing glutathione S-transferase, calmodulin-related calcium sensor protein, an EF-hand transcription factor, histidine-containing phosphotransfer protein and a zinc-finger protein) have been chosen for further functional studies using overexpression and RNAi knockout techniques. Whole plant physiology analyses conducted with a Li-COR 6400 portable photosynthesis system under defined conditions (light intensity of 1500 µMol/m^2/second, temperature of 25°C, and CO_2 concentration of 380 ppm) indicated that switchgrass cv. Alamo inoculated with PsJN had consistently lower transpiration, lower leaf conductance, and higher water use efficiency compared to non-inoculated control plants. A study investigating gas exchange throughout the course of development is on-going. Leaf gas exchange is being measured weekly in cohorts of leaf ages as the plants mature. Our laboratory has also established a lignin analytical method and a lignocellulose saccharification method. In order to develop a low input and sustainable switchgrass feedstock production system utilizing a beneficial bacterial endophyte, we assessed growth performance of PsJN-inoculated plants in unfertilized field soil and grown in a greenhouse under ambient conditions in the fall of both 2010 and 2011. In both years PsJN-inoculated plants produced significantly higher biomass compared with non-inoculated controls, demonstrating the potential benefit of switchgrass bacterization with PsJN, for switchgrass cultivation on marginal lands.

Broad Impacts:

The microarray data generated during this project have been incorporated into the switchgrass GeneAtlas database at the BESC (the DOE BioEnergy Science Center) via the Genomics Core Facility at the Noble Foundation, which will benefit the entire switchgrass research community. Elements of this project have also been integrated into local K-12 and undergraduate education programs. These have included regional Piedmont Governor's high school students, and seniors from Averett University (Danville, VA), who have conducted research internships associated with this project. This collaboration fosters public understanding of the use of beneficial endophytes to improve bioenergy crop yield and its sustainability. The project has been developed in collaboration with regional partners, i.e., Lynchburg Grows, a non-profit urban farm and environmental educational center that provides job training and education for over 2,000 youth-at-risk annually, as well as mentally- and physically-handicapped individuals in the field of agriculture (www. lynchburggrows.org).

Deliverables:

Publications:

"Growth promotion and colonization of switchgrass (*Panicum virgatum*) cv. Alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN", submitted for publication on June 30, 2011 to the journal Biomass and Bioenergy (under review).

Oral/ Poster Presentations:

Invited talk: "Development of switchgrass (*Panicum virgatum* L.) for marginal lands based on genotypic compatibility with beneficial bacteria" was presented in Track 2-1: Feedstock Genomics and Plant Biotechnology Development during the First Annual World Congress of Bioenergy held in Dalian, China, April 25-29, 2011.

Invited talk: "Development of a low input switchgrass production system harnessing beneficial bacterial endophytes" was presented in 2011 International Biomass Conference and Expo held in St. Louis, May 1-4, 2011.

Invited talk: "Genetic enhancement of biofuel and bioenergy crops" was presented in the Third International Symposium on Jerusalem artichoke held in Yinchuan, China, August 20-24, 2011.

Invited talk: "Mechanisms of switchgrass Alamo growth promotion by the beneficial bacterial endophyte *Burkholderia phytofirmans* strain PsJN" will be presented in 2012 Plant and Animal Genomics XX to be held in San Diego, January 14-18, 2012.

A selected lunchtime speaker by 2011 Genomic Sciences Meeting Student Travel Grant: "The Beneficial Bacterial Endophyte *Burkholderia phytofirmans* Strain PsJN Significantly Promotes Switchgrass Alamo Growth was presented in the 2011 Genomic Science Annual Contractor-Grantee Meeting/USDA-DOE Plant Feedstock Genomics for Bioenergy Program Meeting, April 10-13, 2010, Crystal City, VA.

Community Resources Generated:

This supported research project was presented by team members to various USDA Program Leaders in Washington DC, Averett University biology students, Chatham Hall Girls Academy biology students, the Danville Rotary Club, Danville Boy Scout Troop 374, as well as local George Washington High School sophomore and senior students.

Training:

Alejandra Lara-Chavez, Postdoctoral Scientist is being trained for molecular biology and analytical chemistry; Scott Lowman, Ph.D. student is being trained in molecular biology, switchgrass tissue culture and transformation, and plant-endophyte interaction; Bingxue Wang, Ph.D. student is being trained in plant ecophysiology, especially in leaf gas exchange and drought tolerance; Mira Tissari, undergraduate student senior, has been trained in switchgrass-endophyte interaction and switchgrass tissue culture; Bethany Gregory, undergraduate student, has been trained in use of the Li-COR 6400 Portable Photosynthesis System, PMS pressure chamber for the measurements of photosynthesis rate, leaf transpiration, leaf conductance and leaf water potential; and Maggie MacLeish, Piedmont Governor's School for Mathematics, Science & Technology, was trained in switchgrass-endophyte interaction.

Collaborations:

Yuhong Tang, Manager, Genomics Core Facility, Samuel Roberts Noble Foundation, Ardmore,

OK
Dr. Guichuan Hou, Director, the Dewel Microscopy Facility, Appalachian State University, Boone, NC
Michael G. Van Ness, Esq., the Executive Director at Lynchburg Grows, Lynchburg, VA
This research was supported by the Office of Science (BER), U.S. Department of Energy

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Modulation of Phytochrome Signaling Networks for Improved Biomass Accumulation Using a Bioenergy Crop Model

Todd Mockler

Project Director: Todd Mockler, Donald Danforth Plant Science Center, tmockler@danforthcenter.org

Co-PDs: Sam Hazen, University of Massachusetts Amherst, hazen@bio.umass.edu

Objectives and Accomplishments:

Light-quality, in particular red and far-red light, and presumably phytochrome signaling, has the potential to profoundly affect biomass accumulation in grasses. Despite the long-known importance of photoreceptor signaling in plant development and growth, relatively little attention has been paid to the identification of phytochrome signal transduction components in grasses. Genomics and systems biology approaches have the promise to associate genetic components with specific phenotypes. Thus we propose to take an integrative systems biology approach to identify candidate phytochrome signaling genes in *Brachypodium distachyon* and will manipulate their expression *in planta* with the aim of modulating important bioenergy traits, namely shoot biomass, yield, and quality. Targeted application of a high-throughput yeast one-hybrid system to candidate transcription factors and fragments of red and far-red light responsive *B. distachyon* promoters will allow us to infer features of phytochrome regulated transcriptional regulatory networks. This project will yield verified ORF clones and transgenic misexpression lines that will be useful resources for the community and valuable for other studies. These approaches are enabled by the development of *B. distachyon* as a bioenergy model in recent years. The specific objectives of the proposal are: a) misexpression of ~180 candidate *B. distachyon* phytochrome signaling genes, including transcription factors implicated in regulation of gene expression by red and far-red light; b) transgenic *B. distachyon* misexpression lines generated in Objective 1 will be subjected to phenotyping. Plants will be grown in various light conditions and morphological and developmental phenotypes, dry biomass accumulation, and seed yield will be scored; c) *B. distachyon* lines exhibiting clear differences in growth and/or growth rates in Objective 2 will be further characterized using a *Clostridium phytofermentans* based bioassay to measure the impact of transgenic manipulation of the candidate *B. distachyon* phytochrome signaling genes on cell wall quality, as

measured by digestibility; and d) direct interactions between candidate phytochrome signaling transcription factors and target promoters will be assessed using a high-throughput yeast one-hybrid system. This system will interrogate interactions between ~20 B*. distachyon* red and far-red responsive *B. distachyon* promoter regions and ~100 *B. distachyon* transcription factors. The results from this analysis will enable modeling of grass phytochrome signaling transcriptional regulatory networks.

Broad Impacts:

This project may identify grass genes instrumental for improving important bioenergy traits, namely shoot biomass, yield, and quality.

Functional Analysis of Regulatory Networks Linking Shoot Maturation, Stem Carbon Partitioning, and Nutrient Utilization in Sorghum

Stephen Moose

Project Director: Stephen Moose, University of Illinois at Urbana-Champaign, smoose@illinois.edu.

Co-PDs:

Patrick Brown, University of Illinois at Urbana-Champaign, pjb34@illinois.edu

Max Moehs, Arcadia Biosciences, Seattle, WA, max.moehs@arcadiabio.com

Objectives and Accomplishments:

Genetic variation in the regulatory pathway controlling shoot maturation impacts many important traits for sustainable biomass production and conversion efficiency to bioenergy. These traits include perennial versus annual growth cycle, plant architecture, flowering time, vegetative senescence, carbon partitioning, cell wall composition, and nutrient use efficiency. Molecular genetic analyses conducted in maize and Arabidopsis have recently identified a conserved regulatory network of interacting genes that control shoot maturation. The microRNA *miR156* acts during early shoot development to repress the expression of specific *SQUAMOSA BINDING PROTEIN-LIKE* (*SPL*) genes that function to promote shoot maturation. The *SPL* genes then activate *miR172*, which downregulates the expression of *APETALA2* (*AP2)*-class transcription factors that suppress both vegetative and reproductive phase change. This project brings together scientists with expertise in maize shoot maturation genes (Moose), sorghum genetics (Brown) and sorghum biotechnology (Arcadia Biosciences) to increase our understanding of

shoot maturation pathway genes in Sorghum and test their utility in improving Sorghum as a bioenergy feedstock.

The three primary objectives of this project are to 1) characterize allelic variation within Sorghum for shoot maturation genes, 2) test for associations between allelic and expression variation for shoot maturation genes with traits important to Sorghum as a bioenergy crop, and 3) evaluate the phenotypic performance of transgenic Sorghum with altered expression of shoot maturation genes. During the first year of the project, we have made progress towards each of these objectives.

The sequenced genome of Sorghum line Tx623 harbors nine *miR156*, six *SPL*, six *miR172*, and four *AP2* genes. Because it is the smallest gene family and functions in the terminal step within the shoot maturation regulatory pathway, we have initiated our analysis of allelic variation in Sorghum with the AP2 class genes. Primer sets for targeted resequencing have been designed and validated with Tx623 DNA, and a survey of allelic diversity in a core set of diverse Sorghum genotypes is ongoing.

We will employ an association genetics approach to assess the functional contributions of allelic variation in shoot maturation pathway genes to key traits of flowering time, stem sugar concentrations, and onset of senescence. Seed increases were conducted during the 2011 growing season for an association panel of 800 genotypes that includes sweet, grain and forage types. Plant height and flowering time phenotypes were measured on the summer 2011 plantings. A subset of 200 of the 800 genotypes was grown in a tropical environment in the winter of 2011 and is currently growing in the winter 2012 nursery. Comparison of flowering times between tropical and temperate environments for these 200 genotypes will allow the separation of photoperiodic or other control of variation in flowering time. Hybrid seed was generated in the summer of 2011 for most of the 800 genotypes crossed with Tx623. These hybrids will be used to validate suspected *cis*-regulatory QTL.

Prior work of PI Moose has established that overexpression of the maize Glossy15 gene in maize delays shoot maturation in a dosage-dependent manner. These Glossy15 overexpressing (Gl15-OX) lines exhibit delayed flowering and senescence, but also increases in total biomass and stem sugar concentrations. Importantly, field trials demonstrated that maize Gl15-OX hybrids maintain high biomass yield potential in low nitrogen soils, indicating an unexpected advantage in nitrogen use efficiency. Our third objective is to extend these findings to Sorghum by increasing activity of the Sorghum Glossy15 gene (SbGl15). Transformations are in progress.

Broad Impacts: The finding that variation in AP2 genes can achieve some of the beneficial aspects of faster dry-down and improved cell wall composition, without the negative pleiotropic effects of more upstream regulators on biomass yields, offers a novel opportunity to both better understand the control of desirable traits, and may provide new strategies for Sorghum improvement via molecular breeding approaches.

Deliverables:

Community Resources Generated: Seed for the 800 genotypes in the association panel will be made available in limited quantities for research purposes.

Training:

Graduate Students: Payne Burks and Brandon James at University of Illinois.

Technical personnel: Mike Steine, Quyen Lam, Jos van Boxtel at Arcadia Biosciences

Collaborations:

Arcadia Biosciences is collaborating with Dr. Peggy Lemaux to evaluate her improved sorghum transformation methods for this project, particularly with sweet sorghum genotypes.

Genomics of Energy Sorghum Biomass Accumulation

John Mullet

Project Director: John Mullet, Texas A&M University, jmullet@tamu.edu

Co-PDs:

Patricia Klein, Texas A&M University

William Rooney, Texas A&M University

Objectives and Accomplishments:

The overall goal of the proposed research is to identify the genetic and biochemical basis for increasing the yield and improving the composition of high biomass cellulosic energy sorghum. The specific objectives of the proposed research are to; (1) characterize the molecular diversity of 750 photoperiod sensitive (late flowering) energy sorghum germplasm accessions and use this information to select ~200 diverse accessions for analysis of variation in stem biomass yield, structure, and composition, (2) map QTL for stem biomass yield, structure, and composition in three energy sorghum populations derived from diverse sorghum parental genotypes, and (3) develop information and biological resources that will enable positional cloning of QTL/genes and analysis of gene regulatory networks that modulate energy sorghum biomass yield, stem structure, and composition.

High biomass energy sorghum (*Sorghum bicolor* L. Moench) has excellent potential as an annual hybrid bioenergy crop. Research on this species will also provide fundamental information about the genetics and genomics of C4 grass energy crop design. Sorghum, like Miscanthus and energy cane, is a highly productive, drought tolerant C4 grass that under optimum conditions can produce ~20dT of biomass per acre making it one of the most productive bioenergy crops currently under development. Sorghum also

has diploid genetics, a relatively small genome (~800Mbp) and a complete genome sequence providing a good technology platform for conducting genome-scale research into pathways that influence biomass yield and composition. Information and biological resources generated by this project will be used to create improved versions of high biomass energy sorghum and other C4 bioenergy grasses in order to minimize acreage used for biomass production, reduce food vs. biofuels competition, reduce the cost of feedstock, while increasing the carbon balance of biofuels and create a sustainable source of biomass feedstock for large scale biofuels production in the U.S.

PROGRESS (Yr 1):

Energy Sorghum Phenology: A time course of biomass accumulation in late flowering energy sorghum hybrids was collected and compared to grain sorghum. Energy sorghum hybrids produced approximately twice as many leaves (20 vs. 46), had longer stems, and accumulated 2.5X more biomass than grain sorghum hybrids due primarily to delayed flowering and increased duration of vegetative growth. Single plant measurements (n=9 per time point) showed that energy sorghum hybrids have the genetic potential to accumulate ~20 dT/acre under irrigated conditions. Variation in biomass composition during the season was assessed using NIR (NIR calibration curves were generated through a separate collaboration involving TAMU/NREL).

Energy Sorghum Germplasm Screening: Approximately 750 photoperiod sensitive late flowering sorghum accessions were selected for analysis of diversity and energy traits. DNA from each line was analyzed using Digital Genotyping on an Illumina GAIIx. Diversity analysis involved comparison of ~18,000 unique 72bp sequences derived from each accession. More than 20,000 SNPs or small INDELS were detected among the accessions analyzed. The diversity of the accessions was analyzed using Structure and other methods for analyzing genetic relatedness. Accessions clustered based on geographic origin and sorghum race. All accessions were grown in small plots in College Station in 2011. Three plants were harvested from each plot in mid-September and analyzed for flowering status, stem length, fresh weight, dry weight, lodging, and biomass composition. Most accessions had not flowered by mid-September, however, variation in other biomass traits was extensive. Approximately 200 of the accessions were selected for seed generation this winter to enable replicated field studies in 2012. Populations are being generated to enable QTL analysis of energy traits and to inform association studies.

Identification and Genetic Characterization of Maize Cell Wall Variation for Improved Biorefinery Feedstock Characteristics

Markus Pauly

Project Director: Markus Pauly, University of California Berkeley, mpauly69@berkeley.edu

Co-PDs: Sarah Hake, Plant Gene Expression Center, hake@berkeley.edu

Project website: http://pmb.berkeley.edu/profile/mpauly

Objectives and Accomplishments: The objectives of this program are to 1) characterize novel maize mutants with altered cell walls for enhanced biorefinery characteristics and 2) find quantitative trait loci (QTLs) related to biorefinery characteristics by taking advantage of the genetic diversity of maize.

Both projects are well in progress. Several chemically mutagenized maize lines have been identified with altered hemicellulose compositions. One of the lines, *candy leaf1* (*cal1*), has been further characterized. *cal1* mutants exhibit a 240% increase of hemicellulosic glucan. This glucan was identified to be a non-crystalline β-1,3-1-4-mixed-linked glucan that leads to 35% increase in saccharification yield when the corn stalk and leaf material were subjected to a standard digestion assay. The maize genome contains 2.5 billion base-pairs, and we were able to find the single point mutation that is responsible for the observed high glucan content. The mutation destroys the active site of a lichenenase, a plant enzyme that usually degrades this mixed-linked glucan.

Taking advantage of the Nested Assocation Mapping (NAM) population, QTLs were established for hemicellulosic glucan content and saccharification yields. Interestingly, those QTLs do not overlap, nor do they contain the *cal1* locus.

Broad Impacts: A novel non-transgenic maize plant (*cal1*) has been identified, whose stover (leaves and stalk) contain more glucan in their walls leading to a higher saccharification yield, when subjected to a standard enzymatic digestion cocktail. Hence, *cal1* biomass provides an excellent feedstock for the biofuel industry. Field trials are in progress.

Deliverables:

Oral/ Poster Presentations:

- Kraemer F, Hake S, **Pauly M**, Characterization of novel cell wall mutants in maize, 2011 Plant and Animal Genome Conference, San Diego, January 15-19, 2011 **(invited speaker)**

- Kraemer F, Thomas T, Hake S, **Pauly M**, Characterization of novel cell wall mutants in maize, 2011 USDA-DOE Plant Feedstock Genomics for Bioenergy Program meeting, Crystal City, April 10-13, 2011 (**Poster**)

- Kraemer F, Hake S, **Pauly M**, Characterization of novel maize cell wall mutants, 2nd Pan American Congress on Plants and BioEnergy, Sao Pedro, Brazil, August 8-11, 2010 (**oral presentation**)

Community Resources Generated:

Pauly M, Kraemer F, Hake S, 2011, Maize variety and method of production, US-patent application No: 13/152,219

Pauly M, Kraemer F, Hake S, 2011, Plants with elevated levels of glucan, US-patent application No: 61/492,769

Training:

- **Florian Kraemer** (graduate student): Identification and characterization of *cal1*

- **Thomas Thomik** (graduate student): QTL analysis of glucan and saccharification in maize

- **Grace Kayser** (undergraduate student): mapping of *cal1*

- **China Lunde** (Lab manager): Mapping of *cal1* and allele identification

Collaborations:

The specific expertise and interaction between the labs of the two PDs also involved collaborations in other areas such as represented by the following publication: Chuck G, Tobias C, Kraemer F, Sun L, Li C, Arora R, Singh S, Dibble D, Vogel J, Simmons B, **Pauly M**, **Hake S**, 2011, Overexpression of the maize conrgrass1 microRNA gene prevents flowering, improves digestibility and increases starch content of biofuel crop plants, **Proceedings of the National Academy of the USA** 108 (42) 17550-17555.

The Hunt for Green Every April: Factors Affecting Fitness in Switchgrass

Gautam Sarath

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Co-PDs: Kenneth P. Vogel, USDA-ARS, Lincoln, NE, Ken.Vogel@ars.usda.gov; Christian M. Tobias, USDA-ARS, Albany, CA, Christian.Tobias@ars.usda.gov; Madhavan Soundararajan, University of Nebraska at Lincoln, NE, msoundar@unlnotes.unl.edu; Paul Twigg, University of Nebraska at Kearney, NE, twiggp@uk.edu.

Objectives and Accomplishments:

Specific Objective 1: Transcript Profiling, Metabolomics, and C and N Partitioning and Recycling in Crowns and Rhizomes of Switchgrass over two growing seasons.

Objectives have been largely met. Plants labeled with 13C and 15N have been harvested.

~ 1 million sequences obtained from cv Summer crowns + rhizomes using the 454 platform was used to assemble a preliminary crown + rhizome transcriptome. **A manuscript describing this dataset has been accepted in the journal Bioenergy Research.** A hybrid "master" transcriptome assembly using open source assembly programs has been built using ~110 million Illumina reads and ~1.3 million 454 data. **Some of this data will be presented at the upcoming PAG Meetings in San Diego (January 2012).**

Specific Objective 2: Gene Profiling During Regreening and Dormancy of Bulked Segregants.

Objective to be completed in late 2012. Plants have been fully established in the fields as clonal replicates.

Specific Objective 3: Analysis of the Extent of LD and Haplotype Diversity Within Kanlow N1, Kanlow, Summer, and Kanlow x Summer.

Plants for this objective were sampled in July 2011; genomic DNA was extracted, normalized and arrayed in plates. Thus far, reduced representation libraries have been constructed from approximately 25% of the samples by digestion with PstI, barcode IDs ligation, pooling, amplification, and sequencing using the Illumina HiSeq2000 platform.

Broad Impacts:

- A detailed understanding of below-ground physiology of switchgrass plants.
- It is likely that future data mining of the transcriptomics studies could reveal other unanticipated insights into switchgrass metabolism.
- Insights into flowering and interactions with crown/rhizome metabolism.

Deliverables:

Publications:

Palmer NA, Saathoff AJ, Kim J, Benson A, Tobias CM, Twigg P, Vogel KP, Madhavan S, Sarath G (2011) Next generation sequencing of crown and rhizome transcriptome from an upland, tetraploid switchgrass. Accepted Bioenergy Research 11/28/2011.

Five other publications acknowledging partial DOE Feedstock Grant support.

Book Chapter:

Bartley LE, Wu Y, Saathoff AJ and Sarath G (2011) Switchgrass genetics and breeding challenges. In M.C. Saha and J. Bouton (Eds.). Wiley and Sons. New York

Oral/ Poster Presentations:

Dr. S. Madhavan, 2011 International Conference on Bioscience, Biochemistry and Bioinformatics, Singapore, February. **(Keynote Address**)

C. Tobias, 2010 Trisocieties Meeting, Genomics Symposium, Long Beach, CA, October.

Sarath et al, 2010, 32th Symposium on Biotechnology for Fuels and Chemicals, Clearwater FL, May.

Sarath et al, 2011, XIX PAG Conference, San Diego, January.

Community Resources Generated:

All 454 sequencing data files have been deposited in the Short-Read Archives of the NCBI.

Training:

Undergraduate: Jonathan Chalky and Brent Moravec — field collections, sorting and grinding of plants, PCR, RNA, protein and DNA isolations. Labeling of field plants with stable isotopes. Plant phenotyping.

Graduate: Nathan A. Palmer: HTS sequencing, data analysis, transcriptome assembly, RNA-Seq, data interpretation, molecular, biochemical and metabolomic studies (454 Sequencing paper).

Post-Doctoral: Aaron J. Saathoff and Hugh Young: Enzymology, metabolomics, genomics, HTS data interpretation, statistical analysis, collation and insights into diverse data sets (several manuscripts including 454 sequencing).

Systems View of Root Hair Response to Abiotic Stress

Gary Stacey

Project Director: Gary Stacey, University of Missouri, staceyg@missouri.edu

Co-PDs:

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Dong Xu, University of Missouri, Columbia, MO
David Koppenaal, EMSL, Pacific Northwest National Laboratory, Richland, WA
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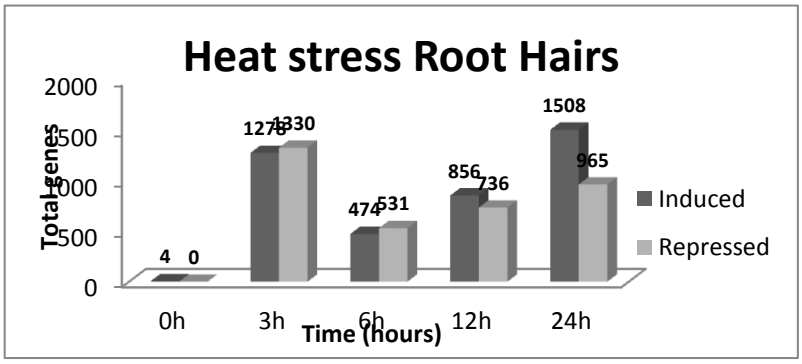
Project website: soyroothair.org/

Objectives and Accomplishments:

Analyze the transcriptional response of root hair cells under conditions of abiotic



stress (i.e., heat and drought stress). We have established conditions for applying heat and drought stress conditions to soybean seedlings in a way that supports the isolation of significant quantities of isolated root hair cells for analysis. Our first RNA seq experiments have been performed profiling the root hair transcriptional response to heat stress. The total number of soybean genes induced during heat stress at the various treatment times are shown in included figure. We are now conducting similar experiments on drought stressed tissues.

2. Analyze proteomic and metabolomic changes in root hair cells under conditions of abiotic stress (i.e., heat and drought stress). These experiments are underway focusing on the membrane associated proteome after application of abiotic stress.

3. Utilize the tools and information developed in Objectives 1-2 to develop descriptive models of root hair metabolic function. We have established the SoyKB web resource (http://soykb.org/) to serve as an integrated tool for soybean functional genomics. The site provides for data storage, integration and analysis.

Broad Impacts:

Over the last 100 years, the atmospheric concentration of carbon dioxide has dramatically increased, in major part due to the burning of fossil fuels, recent rapid industrialization, and land use changes. The predicted effects of continued climate change are complex but include effects on air and surface temperature, with coincident effects on water availability. Soil temperature can influence root growth, cell elongation, root length and extension, initiation of new lateral roots and root hairs, and root branching. These effects are likely manifestations of the variety of physiological effects brought about by temperature on plant roots, including effects on water availability. In order to properly understand the effects of climate change, predictive models must be developed based on accurate experimental data. Systems biology seeks to address these needs by providing a comprehensive, quantitative analysis of the manner in which all the components of a biological system interact functionally over time and space. The ultimate goal is a new, predictive view of biological function, supplanting the older descriptive understanding. However, challenges remain before this goal can be achieved. For example, integration of dissimilar data (e.g., proteomics, metabolomics, transcriptomics, etc.) remains a formidable challenge; this problem is compounded by the issue of “signal dilution” where most studies average the response of whole tissues, obscuring the actual cellular response. Approaches are needed to conduct functional genomics on single cells. Our vision is to utilize the soybean root hair system to explore, at a systems level, the biology of a single, differentiated plant cell type, while gaining novel insight into the

impacts of temperature and water availability on a crucial root cell necessary for nutrient uptake. The proposed research should provide unambiguous measurements of the impact of these environmental factors on plant cell function, without the compounding effects of tissue dilution. The data obtained will allow the development of computational models to examine regulatory networks that function at a single cell level to control the response to environmental change. Ultimately, we hope to provide a better understanding of the impacts of climate change on plant root physiology. In addition to the expected research outcomes, the project will also provide training for graduate and postdoctoral students to prepare them for their future careers.

Deliverables:

Publications:

Trupti Joshi, Kapil Patil, Michael R. Fitzpatrick, Levi D. Franklin, Qiuming Yao, Zheng Wang, Marc Libault, Laurent Brechenmacher, Babu Valliyodan, Xiaolei Wu, Jianlin Cheng, Gary Stacey, Henry Nguyen and Dong Xu. Soybean Knowledge Base (SoyKB): A Web Resource for Soybean Translational Genomics. BMC Genomics. In press.

Z. Wang, X. Zhang, M. Le, D. Xu, G. Stacey, and J. Cheng. A Protein Domain Co-Occurrence Network Approach for Predicting Protein Function and Inferring Species Phylogeny. PLoS ONE. 6(3): e17906, 2011."

Oral/ Poster Presentations: 1. Citation as the publication above, Presented at the Tenth Asia Pacific Bioinformatics Conference in Melbourne, Australia. 17-19 Jan, 2012.

Community Resources Generated: SoyKB, http;//SoyKB.org, a web resource for soybean functional genomics.

Training: Dr. Nicolas Gomez-Hernandez, Postdoctoral Associate
Dr. Mingzhu Zhu, Postdoctoral Associate
Trupti Joshi, Ph.D. Student
Ning Zhang, Ph.D. Student
Jiguang Wang, Ph.D. Student

Phenomic Analysis of Natural and Induced Variation in Brachypodium distachyon

John Vogel

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Objectives and Accomplishments:

Objectives: **1)** Assemble a collection of natural accessions and 2,000 homozygous T-DNA lines. **2)** Conduct a detailed phenotypic characterization of the collection using a phenomic approach. **3)** Begin detailed characterization of a select group of mutants and natural accessions.

Accomplishments:

We have completed optimizing growth conditions and have begun high-throughput phenotyping. Variables optimized include: soil type, nutrient levels, light intensity, daylength and vernalization requirements. Significantly, we saw up to a 3-fold difference in photosynthetic rate among 12 natural accessions in preliminary trials. We also saw a 2-fold difference in relative growth rate and 10-fold difference in final biomass. We constructed nitrogen and phosphorous response curves to select nutrient limiting levels for the final experiments. We investigated the effect of daylength on plant health and generation time and selected 16 hour days for the main experiment. The final parameter analyzed was the effect of vernalization on flowering time with the goal of identifying vernalization times for each line such that they all flower at approximately the same time after being placed into the growth chamber. This will allow an 'apples to apples' comparison that will not be overwhelmed by flowering time. With the optimization completed, experiments to phenotype 160 natural accessions were initiated in September and we expect to have the final dataset by mid December.

Since the inception of the project we have gained access to additional germplasm resources. First, a collaborator, Luis Mur, has collected over 1,000 lines from Spain. Second, David Garvin has developed a F_6 RIL population of 146 lines. Since we have already genotyped this population it is possible to immediately identify QTLs for any phenotype examined. We have added both these valuable collections to the project for phenotyping.

Another part of the project is to create homozygous T-DNA lines for phenotypic analysis. We are using a PCR-based approach to identify homozygous individuals in the M1 generation (the first segregating generation). Starting with 5,348 flanking sequence tags (FSTs) identified 1,655 insertions within genes and another 1,268 insertions that may disrupt expression. We genotyped ~14,480 M1 individuals from 1,480 T-DNA lines and have harvested tissue from 520 more lines for genotyping. We have identified putative homozygous M1 individuals from 984 lines. Of the 610 putative homozygous lines examined in the next generation, 365 were confirmed homozygous and 240 of those have been shipped to Australia for phenotyping. Assuming a similar efficiency for the lines remaining to be

processed, we expect to identify ~720 homozygous lines from this first batch. This is less than our goal of 2,000 lines because the overall efficiency of identifying homozygous lines is lower than anticipated. No one factor seems to be responsible, rather, a percentage of lines seem to fall out at each step. We have identified two significant factors that we can address. First, the starting pool of lines with insertions in genes was smaller than anticipated largely because there was a greater than expected tendency for T-DNAs to insert close to, but not in, genes. Second, a percentage of the FSTs may be mis-mapped to the genome due to poor FST sequence quality or gene family members. The Illumina-based method we have adopted to identify additional FSTs will address both of these problems (see our T-DNA project progress report for details). Going forward, we will select a second set of lines to screen for homozygous individuals from the next set of FSTs that will be generated in the next 2 months. However, since we are effectively halfway through the project, we may not reach the original goal of 2,000 homozygous lines. In this context we are fortunate to have added the additional natural accessions and RI population (at least 350 lines) mentioned above.

Broad Impacts:

This project has helped establish Brachypodium as a model system and has been instrumental in attracting additional funding.

Deliverables:

Oral/ Poster Presentations:

Opening talk and a plenary talk “Natural diversity from genomics to phenomics” at the First European Brachypodium Workshop. Versailles, France. This meeting was a milestone because it is the first international meeting focused on Brachypodium. 2011

Invited seminars at several institutions including: Max Planck Institute for Plant Breeding Research, Germany; Seoul National University, Korea; Oregon State University; Ohio University; Ohio State University; and Cornell University.

Lectures at plant courses: Practical Summer Workshop in Functional Genomics, Ohio State University; Cold Spring Harbor Plant Methods Course (2 years).

Community Resources Generated:

365 Homozygous T-DNA lines. The community will be able to order seeds through our website http://brachypodium.pw.usda.gov/ as soon as we have bulked them up.

Training:

Two postdoctoral researchers have been trained during this project. Jennifer Bragg has made the homozygous T-DNA lines and Richard Poire has done the phenomic work. One lab technician, Amy Anderton, has contributed to the project.

Collaborations:

A companion project with co-PI Michelle Watt to examine root traits in greater depth was funded by the Australian Grain Development Council. A project to resequence 56 natural accessions through the DOE JGI CSP was initiated. Collaborations to examine pathogens on the collections were initiated with Michael Ayliffe, CSIRO, Canberra, Australia, and Tim Fitzgerald and John Manners, CSIRO Plant Industry, Queensland, Australia. Collaborations with Luis Mur, University of Aberstwyth, UK, and David Garvin were initiated to phenotype their germplasm.

Insertional Mutagenesis of Brachypodium distachyon

John Vogel

Project Director: John Vogel, USDA-ARS Western Regional Research Center, john.vogel@ars.usda.gov

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Gerard Lazo, USDA-ARS Western Regional Research Center, gerard.lazo@ars.usda.gov

Olin Anderson, USDA-ARS Western Regional Research Center, olin.anderson@ars.usda.gov

Project website: http://brachypodium.pw.usda.gov/

Objectives and Accomplishments:

Objectives for renewal project: **1)** Generate 30,000 insertional mutants. **2)** Sequence DNA flanking the insertion sites of all mutants. **3)** Collaborate with other groups creating T-DNA lines.

Accomplishments (including initial grant):

Our T-DNA collection currently contains 10,338 lines. We have sequenced 7,111 lines to obtain 17,611 sequences. Of these sequences, 8,107 hit the Brachypodium genome and are thus considered flanking sequence tags (FSTs). These FSTs hit 5,348 unique loci. 1,655 insertions are within genes and another 1,286 insertion sites are close enough to genes to possibly disrupt expression.

Our initial FST sequencing used Sanger technology and was state of the art at the time. However, we are now collaborating with Joe Ecker at the SALK Institute to use four-dimensional pooling and Illumina sequencing to generate FSTs. This approach, called TDNA-Seq, is more efficient than Sanger sequencing because there is no sequence interference when multiple T-DNAs are contained in a single line (the average number of insertions per line is ~1.5). Another significant advantage is a huge

reduction in the number of DNA preps required. For 10,000 lines it is only necessary to prepare DNA from 40 pools of 1,000 lines.

Since our group alone will not generate enough T-DNA lines to have a high likelihood of obtaining an FST in any particular gene, we formed the International Brachypodium Tagging Consortium (IBTC) to efficiently pool resources. We are sequencing ~5,300 lines produced by collaborators in the U.S., UK, China and Canada. Tissue has been harvested and DNA is being made for the first set of 8,000 lines.

Broad Impacts:

This project has helped establish Brachypodium as a model for the grasses.

Deliverables:

Publications (selected from six):

Bragg, J. N., **Vogel, J.P.** 2012 High-efficiency transformation of *Brachypodium distachyon* using *Agrobacterium tumefaciens.* **Plant Functional Genomics: Methods in Molecular Biology.** Ed. Springer, P. Humana Press. in press

Brkljacic, J., et. al. 2011. Brachypodium as a model for the grasses: Today and the future. **Plant Physiology** 157: 3-13

Tyler, L. et. al. 2010 Annotation and comparative analysis of the glycoside hydrolase genes in *Brachypodium distachyon.* **BMC Genomics** 11:600

Vogel, J.P. et. al. 2010 Genome Sequencing and Analysis of The Model Grass *Brachypodium distachyon.* **Nature**. 463: 763-768

Oral/ Poster Presentations (selected from many):

Opening talk “Brachypodium’s rise as model” and a plenary talk “Natural diversity from genomics to phenomics” at the First European Brachypodium Workshop. Versailles, France. In addition, a collaborator’s talk “T-DNA mutagenesis in *Brachypodium distachyon*” presented data for the T-DNA tagging project. This meeting was a milestone because it is the first international meeting focused on Brachypodium. 2011

Invited talk “*Brachypodium distachyon:* a new model to study the grass cell wall” at the Berkeley Synchrotron Infrared Structural Biology (BSISB) program workshop during the Advanced Light Source Users' Meeting. Berkeley, CA. 2010

Plenary talk “*Brachypodium distachyon*: resource development and applications to study the grass cell wall” at the Canadian Plant Genomics Workshop, Saskatoon, Canada 2009

Plenary talk “*Brachypodium distachyon*: a New Model for Biomass Crops” at the Society for In Vitro Biology meeting. Charleston, SC. 2009

Invited talk "*Brachypodium distachyon*: a new model for the grasses" at the USDA AFRI /NRI plant genome, genetics and breeding project directors meeting. San Diego, CA 2010

Invited talks at a workshop and a satellite meeting at the American Society of Plant Biology meeting, Honolulu, HI, 2009

Invited seminars at several institutions including: Max Planck Institute for Plant Breeding Research, Germany; Seoul National University, Korea; Oregon State University; Ohio University; Ohio State University; Cornell University;

Lectures at plant courses: Practical Summer Workshop in Functional Genomics, Ohio State University; Cold Spring Harbor Plant Methods Course (2 years).

Community Resources Generated:

10,338 Brachypodium T-DNA lines created (http://brachypodium.pw.usda.gov/)

5,348 loci tagged by 8,107 FSTs

As of 11-9-2011 we have sent 119 lines to 24 labs around the world.

Training:

Three postdoctoral researchers have been trained during this project. Jennifer Bragg and Jiajie Wu created and sequenced the first 8,700 T-DNA lines. Mandy Hsia is sequencing lines using T-DNA-Seq and making more T-DNA lines. Three undergraduates, Alana Clark, Allison Soung, and Stephanie Hong, and one lab technician, Rita Nieu, have been trained during this project.

Collaborations:

A phenomics project (see second project progress report) with Robert Furbank and Michelle Watt, CSIRO, Canberra, Australia. Sequencing T-DNA lines from members of the IBTC: Philippe Vain, John Innes Centre, UK; Mark Jordan, Agriculture & Agri-Food Canada; Caixia Gao and Daowen Wang, Institute of Genetics and Developmental Biology, CAS, China; Heidi Keppler, John Sedbrook and Rick Amasino, Great Lakes Bioenergy Research Center, USA; and David Garvin, USDA-ARS/ Univ. of Minnesota. Place FST data in the Brachybase database with Todd Mockler, Donald Danforth Plant Science Center.

Sorghum Biomass Genomics and Phenomics

Jianming Yu

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Objectives and Accomplishments:

We have three project objectives. 1. Genotypically characterize a diverse set of sorghum germplasm for selective phenotyping and phenotypically assess the biomass potential of a selected representative set for yield and composition for genomewide selection (GS). 2. Develop a standardized method for high-throughput and cost-effective phenotyping for sorghum biomass composition through near-infrared reflectance (NIR) spectroscopy. 3. Discover additional useful germplasm by genomewide prediction and useful genes by association mapping for biomass yield and composition.

Part of the Objective 1 has been met. We have selected 1000 biomass sorghum accessions from GRIN and the genotyping-by-sequencing experiment is being carried out.

Broad Impacts:

This strategy employed in this project will generate critical information on how to tap into the vast plant germplasm collections for biomass crop improvement, and how to increase the information contained in genotypic and phenotypic data for the selected germplasm so that this information can generate maximum knowledge to enrich our understanding of the germplasm and genotype-phenotype relationship.

Deliverables:

Publications:

Li, X., C. Zhu, J. Wang, and J. Yu. 2012. Computer simulation in plant breeding. *Advances in Agronomy* 116 (in press).

Oral/Poster Presentations:

Wu, Y., X. Li, W. Xiang, C. Zhu, Z. Lin, Y. Wu, J. Li, G. Bai, S. Bean, M.L. Wang, H. Trick, M. Tuinstra, T. Tesso and J. Yu. 2012. Natural genetic variation at Tan1 defines tannin in sorghum grain and offers seedling cold tolerance. *Plant and Animal Genome XX Conference*.

Li, X., C. Zhu, Z. Lin, Y. Wu, D. Zhang, G. Bai, W. Song, J. Ma, G. Muehlbauer, M. Scanlon, M. Zhang, and J. Yu. 2012. The pattern and dynamics of genome and chromosome across species. *Plant and Animal Genome XX Conference*.

Enhancing gene discovery and plant breeding by combining genomic technology and genetic design. Chromatin, Oct. 18, 2011, Lubbock, TX.

Li, X., C. Zhu, J. Wang, and J. Yu. 2011. Computer simulation in plant breeding. *2011 Annual ASA/CSSA/SSSA Meeting*.

Community Resources Generated:

The selection of the 1000 biomass sorghum accessions was shared with the community. Accession name, origin, and the selection process are available upon request. These will be made available at the project website.

Training:

Xin Li, graduate student, Kansas State University, genomewide selection

Chengsong Zhu, postdoctoral research associate, Kansas State University, genomewide selection

Feng Xu, graduate students, Kansas State University, NIR prediction for biomass composition

Collaborations:

Chromatin, Inc. Chromatin is developing specialized sorghum feedstocks for the renewables industry through its subsidiary, Sorghum Partners®. These next generation, high-quality feedstocks are being designed by Chromatin's elite team of experts to meet the precise yield and performance requirements of the bioprocessing industry.

USDA-DOE Feedstock Genomics for Bioenergy Program Nontechnical Summaries of New Awardees

(In alphabetical order)

Association Mapping of Cell Wall Synthesis Regulatory Genes and Cell Wall Quality in Switchgrass

Laura Bartley

INVESTIGATORS: Bartley, Laura E., Wu, Y., Brummer, E.C., Saha, M.

INSTITUTIONS: University of Oklahoma, Oklahoma State University, Samuel Roberts Noble Foundation

NON-TECHNICAL SUMMARY: The goal of this project is to identify natural switchgrass genetic variation that correlates with extreme lignocellulose qualities. Lignocellulose is the material the makes up most of the dry mass of plant leaves, stems, and roots. Such material is being targeted for conversion into biofuel due to its abundance and potential for sustainable production. Switchgrass is an attractive species for development as a biofuel crop as it can grow to a large size and tolerate drought and other stresses. Lignocellulose qualities critically impact the efficiency of conversion of lignocellulose into biofuels, both through so-called biological conversion processes and, to an extent, through thermochemical conversion processes.

OBJECTIVES: (1) Identify grass genes that may control lignocellulose-to-biofuel conversion quality and variants (so-called single nucleotide polymorphisms or SNPs) in those genes within 36 diverse, lowland switchgrass accessions, or families. (2) Measure the association between the identified SNPs and lignocellulose quality in a larger collection made from the 36 accessions. (3) Determine if the identified significant SNP-biomass quality associations also hold in two additional, independent switchgrass populations, representing 110 accessions. This will lead to the identification of switchgrass plants with superior lignocellulose quality for immediate use in breeding programs and SNP markers that may be used to rapidly screen other grass populations for similar traits. (4) Test the functions of selected genes to obtain insight into the genetic control of lignocellulose composition for further biomass improvement.

APPROACH: The objectives will be accomplished through an innovative combination of gene network analysis and advanced breeding techniques, as follows: (1) Gene networks from the reference grass, rice, and other data will be used to identify candidate control genes. The DNA for these genes will be captured from diverse switchgrass plants and sequenced to identify SNPs. (2 and 3) In the larger populations, lignocellulose quality will be rapidly determined based on light absorption (NIRS) and wet lab analyses. Selected SNPs will be detected with a method that handles the genomic complexity of switchgrass. These data sets will be subject to statistical association analysis. (4) The functions of possible lignocellulose control genes will be tested in reference grasses with mutants with increased and decreased gene expression.

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Functional Genomics of Sugar Content in Sweet Sorghum Stems

David Braun

INVESTIGATORS: Braun, D. M., Dweikat, I., Ferrieri, R., Babst, B.

INSTITUTIONS: University of Missouri-Columbia, University of Nebraska-Lincoln, Brookhaven National Laboratory

NON-TECHNICAL SUMMARY: Enhancing the production and conversion of plant feedstocks to utilizable sources of energy will decrease the U.S. dependency on fossil fuels while reducing greenhouse gas emissions. Sweet sorghum is a rapidly growing, high biomass, widely adaptable crop with tremendous potential for biofuel production. Sweet sorghum accumulates very high concentrations of sucrose in the stem, which can be efficiently converted to ethanol. We hypothesize that sucrose accumulation in sweet sorghum can be further improved if we understand the mechanisms regulating carbon allocation to stems. The proposed research will use a combination of approaches, spanning genomics, molecular genetics, biochemical phenotyping, and detailed physiological studies to identify bioenergy-relevant genes and to understand their functions in carbon partitioning in sweet sorghum. The knowledge

obtained through this project will provide valuable information toward improving sweet sorghum for bioenergy.

OBJECTIVES: 1. To map and clone Quantitative Trait Loci (QTLs) related to stem biomass, total biomass, and sugar accumulation in sweet sorghum stems. 2. In order for sucrose to accumulate to high levels within sorghum stem internodes, it must be transported across the plasma membrane, presumably by sucrose transporters (SUTs). We will characterize all SUTs in sweet sorghum to determine their functions. 3. To understand the partitioning of carbohydrates to stem tissue, we will perform comparative phenotyping of the plant materials relevant to aims 1 and 2, using a robust and highly sensitive radiochemical tracer, carbon-11 (^{11}C).

APPROACH: This project will screen a set of recombinant inbred lines (RILs) that were developed from a cross between a sweet and grain sorghum that differ in a number of traits, including total soluble sugar in the stalks, as well as grain and total biomass production. The data generated from this aspect of the project will be useful for the identification of markers linked to genes that control QTLs of economic importance in sweet sorghum. In parallel, quantitative RT-PCR will be used to measure the expression level of all sorghum SUT genes in multiple tissues. RNA in situ hybridization will be used to determine which genes are specifically expressed in stem phloem vs. storage parenchyma cells. Reverse genetic approaches will be used to determine the functions of select SUTs and to identify which ones have critical roles in the stem accumulation of sucrose. Additionally, we will use the short-lived radiotracer ^{11}C, administered as $^{11}CO_2$ to leaves, for phenotyping whole-plant carbon transport dynamics. Concurrently, some destructive harvesting will be conducted to measure biochemical partitioning of ^{11}C to sugars, starch, and cellulose in the source leaves, internodes, and panicles, and to correlate gene function data from objectives 1 and 2 with ^{11}C transport and partitioning characteristics.

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Creation and High-precision Characterization of Novel Populus Biomass Germplasm

Luca Comai

INVESTIGATOR: Comai, L., Groover, A.

INSTITUTION: UC Davis and USFS Davis

NON-TECHNICAL SUMMARY: The overall goal of the proposed research is to provide new genomic tools for plant breeders to rapidly identify poplar germplasms with unique genotypes with increased biomass yields. The proposed research will take advantage of advances in DNA sequencing technology to identify trees from traditional breeding programs that carry additional chromosomal regions. Such properties can confer faster growth, as has been shown in other plant species. Techniques for creating poplar hybrids with unique combinations of chromosomal regions at high frequency will also be developed. The project addresses the specific challenge posed by the long generation time of trees and aims at generating and analyzing diversity through fast and cost-effective non-transgenic genetic manipulation. The development of high yielding bioenergy poplar will decrease the acreage required for biomass production, minimize food vs. biofuels land use trade-offs, and the cost of biomass per dry ton, while increasing biofuels carbon balance and sustainability.

OBJECTIVES: 1. Characterize ploidy changes, deletions and possible rearrangements in existing Populus hybrids, correlate these data with biomass properties. 2. Produce germplasm with enriched genotypic and chromosomal dosage variation compared to traditional Populus hybrids and test the contribution of smaller chromosomal regions to interesting traits. This germplasm will serve as a testbed for exploring correlations between the dosage of specific chromosomal segments and growth traits, and to identify commercially-relevant genomic combinations. 3. Compare these dosage variants to parents and other hybrids and correlate gene expression with biomass-related traits.

APPROACH: The project will explore fundamental features of ploidy changes, dosage and heterosis underlying Populus hybrids and their phenotypes. Previous research has shown that commercial F1 hybrids can be triploid or aneuploid, and can have transgressive phenotypes desirable for biomass production. Unfortunately the chromosomal composition and genetic features of these individuals are poorly characterized, and have not yet been examined by genomics research or effectively manipulated in breeding programs. Next generation sequencing-based approaches will be used to characterize parental species and multiple hybrids. γ−irradiation of pollen will be used to induce deletions of varying size. Selected individuals with interesting genomic characteristics will be subjected to RNA-seq for comparison to other hybrid and parental species.

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Genomic and Breeding Foundations for Bioenergy Sorghum Hybrids

Stephen Kresovich

INVESTIGATORS: Kresovich, S.; Paterson, A.H.; Feltus F.A.

INSTITUTIONS: University of South Carolina; University of Georgia; Clemson University

NON-TECHNICAL SUMMARY: Our goal is to build the germplasm, breeding, genetic, and genomic foundations necessary to optimize cellulosic sorghum as a bioenergy feedstock. We define cellulosic sorghum as an annual or perennial form that is bred and selected to maximize carbon (energy) accumulation per unit time, land area, and/or production input (water, nutrients, pesticides, etc.). The ideotype will be extremely tall, heavy tillering, large-barreled, dry stemmed, photoperiod-sensitive material that aggressively regrows in environments with milder winters (southern U.S.).

OBJECTIVES: The specific objectives of the proposed research are: (1) to develop ten nested association mapping populations (NAMs) and a diversity panel necessary to dissect the genetic bases of carbon accumulation and partitioning of cellulosic sorghum; (2) to phenotype these NAMs and diversity panel for patterns of carbon accumulation and partitioning and to correlate these traits with DNA sequence variation that will underlie future breeding/genetic studies; (3) to lay the foundation for integrating genomic selection and other genomics-based strategies into cellulosic sorghum breeding programs; and (4) to identify and create cellulosic male-sterile (A lines), maintainer (B lines), and restorer (R lines) germplasm necessary to exploit heterosis specifically targeted at energy production.

APPROACH: We will test the hypothesis that parallel evolution of and selection for cellulosic sorghum in different botanical races may have involved convergent mutations or introgressions, with the result of a high level of correspondence of QTL locations in crosses involving different botanical races. This implies that the genetic control of the traits fundamental to this ideotype may be relatively simple, and quickly identified to expedite marker-based selection. We also will implement a systems biology approach toward tests of multiple hypotheses that specific molecular pathways are underlying QTLs sets by fitting *de novo* generated sorghum gene-gene coexpression interactions (and their predicted function) to QTLs on a trait-by-trait basis. In this way, we will identify groups of candidate genes with evidence that makes them priorities for functional validation through association genetics, reverse genetics, or transgenic approaches. Synergistically to this effort, ongoing DOE JGI community sequencing and NSF-BREAD genotyping projects will provide the fundamental information, pipeline, and associated database for us to identify the required SNPs. This project also will complement a NAM set being developed for grain

sorghum, with ten additional populations that address traits of singular importance to cellulosic sorghum, using a common parent ('Grassl') and other lines exhibiting wide variation in genetic background, geographic origin, biomass composition, and agronomic phenotype. Lastly, products (A, B, and R lines) critical to successful breeding of cellulosic hybrids will be developed. Heterosis will impact yield and composition in manners for which we expect to provide diagnostic DNA markers, while simultaneously advancing the crop for use by the U.S. seed industry.

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Modulation of Phytochrome Signaling Networks for Improved Biomass Accumulation Using a Bioenergy Crop Model

Todd Mockler

INVESTIGATOR: Mockler, Todd C.; Hazen, Samuel P.

INSTITUTION: Donald Danforth Plant Science Center

NON-TECHNICAL SUMMARY: Plant growth and development, including stem elongation, flowering time, and biomass yield, are affected by the light environment. The goal of the proposed research is to use genomics and genetics in the model grass system Brachypodium distachyon to identify genes involved in light perception and signaling that will increase the yield and improve the composition of bioenergy grasses. Identification of genes capable of modifying growth characteristics of grasses, in particular increasing biomass accumulation, by modulating light perception and signaling will provide valuable candidates for manipulation in bioenergy grass crops through targeted breeding or engineering efforts.

OBJECTIVES: 1. Misexpress 180 candidate Brachypodium phytochrome signaling genes, including transcription factors implicated in regulation of gene expression by red and far-red light. 2. Screen and score the misexpression lines for morphological and developmental phenotypes, dry biomass accumulation, and seed yield. 3. Characterize lines exhibiting clear differences in growth and/or growth rates using a Clostridium phytofermentans based bioassay to measure the impact of manipulating the expression of phytochrome signaling genes on cell wall quality, as measured by digestibility. 4.

Interrogate direct interactions between 100 candidate phytochrome signaling transcription factors and 20 target promoters using a high-throughput yeast one-hybrid system.

APPROACH: This project will screen a collection of promising phytochrome signaling gene candidates for functions in yield and composition traits and define the transcriptional regulatory networks that modulate these traits. As a first step the expression of genes predicted to function in Brachypodium phytochrome signaling will be systematically manipulated by overexpression and knockdown approaches. The resulting collection of mutant Brachypodium lines will then be screened for phenotypes relating to morphology, stature, biomass accumulation, and cell wall composition. In parallel direct interactions between candidate phytochrome signaling transcription factors and their target promoters will be interrogated using a high-throughput yeast one-hybrid system in order to further elucidate the mechanisms underlying the gene regulatory networks that control light-regulated biomass yield.

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Quantifying phenotypic and genetic diversity of *Miscanthus sinensis* as a resource for knowledge-based improvement of *M.* ×*giganteus* (*M. sinensis* × *M. sacchariflorus*)

Erik J. Sacks

INVESTIGATORS: Erik J. Sacks, Joe Brummer, Megan Hall, Stephen Long, Junhua Peng, Toshihiko Yamada, and Chang Yeon Yu

INSTITUTIONS: University of Illinois, Colorado State University, UC Berkeley, Wuhan Botanical Garden , Hokkaido University, Kangwon National University

NON-TECHNICAL SUMMARY: Improved cultivars of perennial bioenergy crops are needed to reduce U.S. dependence on foreign oil, decrease greenhouse gas emissions, and provide new economic opportunities for farmers. *Miscanthus* is among the most promising cellulosic biofuel crops for

temperate, moist environments, which include nearly all agricultural lands from the central U.S. through to the eastern U.S. However, only a single sterile triploid genotype of *M.* ×*giganteus* (*M. sinensis* × *M. sacchariflorus*) is currently available for feedstock production, which is a serious potential risk because a new disease or pest could conceivably cause extensive damage to plantings. Thus, there is a need to broaden the genetic base of *Miscanthus* for bioenergy. The goal of this project is to obtain fundamental information about *M. sinensis* genetic diversity and environmental adaptation, to facilitate development of *Miscanthus* as a bioenergy crop. We will also identify genes and molecular markers associated with traits of interest, which will improve our knowledge of *Miscanthus* genomics and provide new tools for increasing the efficiency of breeding improved cultivars of *Miscanthus*.

OBJECTIVES: 1. Determine genetic diversity and population structure for a core collection of ~500 *M. sinensis* genotypes. 2. Quantify phenotypic variation in *M. sinensis* for key yield and adaptation traits at field trial sites in the U.S., Canada and Asia, and assess the effects of genotype × environment (GxE) interactions. 3. Identify genes governing key traits.

APPROACH: This project will establish and evaluate a core collection of *M. sinensis* genotypes from throughout the species natural distribution in China, Japan and Korea. Molecular marker analysis of the core collection will be used to elucidate evolutionary relationships among the accessions, which will become a useful aid for parent selection in breeding programs. In parallel with the molecular analysis, the core collection will be grown in replicated field trials at an environmentally diverse set of locations, and evaluated for yield-potential and adaptation. The field trial study will facilitate the breeding of improved cultivars that are adapted to U.S. production environments. A combined analysis of the molecular marker data and the phenotypic data will enable identification of molecular markers associated with traits of interest, which is expected to improve the efficiency of future *Miscanthus* breeding efforts. Lastly, candidate genes of known function in other grasses will be evaluated to determine if they are likely to have a similar function in *Miscanthus*.

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Discovering the desirable alleles contributing to the lignocellulosic biomass traits in *Saccharum* germplasm collections for energy cane improvement

Jianping Wang

INVESTIGATOR: Wang, Jianping; Glynn, Neil; Gilbert, Robert.

COLLABORATORS: Schnell, Raymond; Binder, Joseph

INSTITUTION: University of Florida

NON-TECHNICAL SUMMARY: The southern US with its long growing season and high light intensity is a potentially significant contributor to the national goal of biomass production. In the sub-tropical regions of the US, energy cane (*Saccharum* complex hybrids) holds great potential as a bioenergy feedstock. The goal of this project is to improve energy cane biomass production through genetic and genomic analysis of an evaluated and selected *Saccharum* germplasm collection to identify the genetic components contributing to biomass production. The project will produce a range of foundational genetic resources and genetic makers for energy cane breeders to efficiently develop energy cane cultivars with increased biomass production and reduced input requirement.

OBJECTIVES: 1. Identify a core collection of approximately 250 accessions from the World Collection of Sugarcane and Related Grasses held at USDA-ARS Miami, FL that captures the greatest extent of molecular and phenotypic diversity. 2. Discover desirable alleles contributing to biomass composition in *Saccharum* spp. through association analysis between allelic variability in candidate genes and lignocellulosic biomass components and cell wall composition. 3. Develop energy cane cultivars through marker assisted selection.

APPROACH: For objective 1) phenotypic and genotypic evaluation of all of the accessions in the world *Saccharum* germplasm collection. A set of accessions, representing most of the overall genetic variance of the collection will be selected to form a core collection. For objective 2), allele variants of candidate genes contributing to lignocellulosic biomass will be obtained from amplification and next generation sequencing. Biomass and bioenergy traits will be evaluated on the core collection using field and laboratory measurements. Association analysis between the phenotypic and genotypic variation will be performed to identify alleles associated to targeted biomass traits that can be used for genotype selection. For objective 3), the desirable alleles contributing to biomass will be converted to genetic markers to assist the selection of energy cane breeding lines in the energy cane breeding program. The selected lines will be further evaluated and characterized in the field.

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Acknowledging USDA-NIFA and DOE Support

Proper acknowledgement of your funding in posters, abstracts, project websites, published manuscripts, presentations, press releases, etc. is critical for the success of this program.

If funded by USDA-NIFA, we expect you to use the following language to acknowledge NIFA support, as appropriate:

"This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. XXX-XXXXX-XXXXX from the USDA National Institute of Food and Agriculture."

We also expect that you will use our agency's official identifier logo (http://www.nifa.usda.gov/about/offices/nifa_logo.html) in all of your slide and poster presentations resulting from your AFRI award.

Please alert us of significant findings, publications, news releases, and other media coverage of your work. With your permission, we may highlight your project in a National Impact story or News Release. If your research is featured on the cover of a scientific journal, we can showcase the cover as well. Examples of these publications can be found in our Newsroom (http://www.nifa.usda.gov/newsroom/newsroom.html).

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