

ACCOMPLISHMENTS REPORT
Western Regional Biomass Research Center

May 2014 – October 2015
Submitted by: Steve Naranjo, Acting Coordinator

Locations Reporting:

Akron, Colorado
 Central Plains Resources Management Research Unit

Albany, California
 Crop Improvement and Genetics Research Unit
 Bioproduct Research Unit

Fort Collins, Colorado
 Soil, Plant and Nutrient Research Unit

Logan, Utah
 Forage and Range Research Unit

Maricopa, Arizona
 Plant Physiology and Genetics Research Unit

Parlier, California
 Water Management Research Unit

(1) Feedstock Development

Logan, UT

Hybrid Basin wildrye grass improves rangeland forage production and nutrition. Basin wildrye ranks among the largest native grasses in western North America and can provide useful forage in the early spring growing season. However, its use is limited by its relatively low forage quality and animal palatability when compared to other wildrye and wheatgrasses. In an attempt to increase forage yield and quality, hybrids between basin wildrye and creeping wildrye were developed by a ARS multi-disciplinary team of scientists in Logan, Utah to study the genetic control of these traits and develop improved wildryes for late fall and winter forage. The hybrids produced 36% more forage than did the parents and commercially available wildrye cultivars, and, thus, are gene sources (i.e., genetic stock for use in further breeding) to improve basin wildrye forage yield. This hybrid was used to identify genes and chromosome regions controlling vegetative yield, plant height, flowering, early-season forage quality (protein), and late-season forage quality traits. The molecular tools and progeny derived from this hybrid are being used to improve understanding of the genetic and physiological mechanisms controlling vegetative yield, and fiber, lignin, and protein content. Such information may lead to the development of new low-input perennial grass feedstocks for the western United States.

Molecular marker development and application improves prediction of high performance orchardgrass. Orchardgrass breeders want to know how cultivars are

related so that they can find unique genetic stocks (i.e., plants that possess yield and/or quality traits not present in commercial cultivars) to make crosses (hybrids) that will result in cultivars having added value and yield. The molecular genetic analysis of a diverse array of North American orchardgrass cultivars by an ARS multi-disciplinary team of scientists in Logan, Utah revealed large genetic differences among the cultivars examined. Thus, cultivars could not be segregated into groups. This was not because orchardgrass cultivars have too little genetic variation, but because they have too much. However, DNA markers were not useful in differentiating all cultivars, and, thus, making predictions of hybrid vigor (i.e., degree of increase in yield and quality) will require breeding for increased uniformity within cultivars. Results also indicated that breeders could use DNA markers to conduct more specific selection among existing genetic stocks to improve the species. Molecular markers were made available for use by private and public orchardgrass breeders to improve selection effectiveness and efficiency to reduce the time required for release of improved cultivars.

Maricopa, AZ

CGI-58 regulates oil accumulation and stress response in plants. The production of oil in the leaves and stems of plants offers a potential mechanism to significantly increase the amount of energy-dense oils that can be recovered from biofuel crops. ARS scientists in Maricopa, Arizona, in collaboration with scientists at the University of Guelph and the University of North Texas, identified the CGI-58 gene as a key regulator of oil accumulation in plant leaves that, when disrupted, results in a substantial increase in oil content in plant leaves. Surprisingly, disruption of this gene also altered production of a class of molecules called polyamines, which are associated with plant stress response. These observations raise the interesting possibility of not only increasing oil content in plant leaves, but also improving stress tolerance in engineered crop plants.

Variation in the content and composition of the waxy, protective layer on oilseed crops. *Brassica napus* L. is one of the most important oilseed crops in the world, but the geographic regions in which it may be planted are restricted to cooler, temperate climates because the crop is negatively impacted by heat and drought stress. One of the factors that influences plant tolerance to heat and drought stress is the plant cuticle, which forms the waxy, protective layer on the outer surface of leaves and stems. To determine whether this layer might be a target for improving heat and drought tolerance of *B. napus*, scientists at the Arid-Land Agricultural Research Center, Maricopa, Arizona, in collaboration with scientists at the University of Illinois, West Virginia University, Cornell University, and the Southwest University in Chongqing, China, determined the variability in wax content and composition amongst a diverse population of over 500 *B. napus* lines. The results revealed that there were substantial differences in the content and composition of the wax layer, and statistical analyses revealed that this variation was likely due to differences in the genetic makeup of the plants. These results are informative to crop breeders who are interested in improving the heat and drought tolerance of *B. napus*, which could potentially expand the geographic regions in which this crop can be grown.

Identification of a new gene called SEIPIN that influences oil production in plants. Many of the genes involved in seed oil production in plants are known, but genes that regulate oil accumulation in non-seed tissues such as leaves and stems are poorly understood. Scientists at the Arid-Land Agricultural Research Center, Maricopa, Arizona in collaboration with scientists at the University of North Texas, the University of Guelph, and the University of Texas Southwestern Medical Center at Dallas identified a gene called SEIPIN that plays a key role in the formation of “lipid droplets”, which are the subcellular organelles in plant cells that house oil within them. Over-expression of SEIPIN in the model plant *Arabidopsis* resulted in a significant increase in oil content not only in plant leaves, but also in plant seeds. These studies identify a new gene that regulates oil accumulation in plants that will be important not only to scientists interested in increasing oil content of plant vegetative biomass, but also for those scientists focused on increasing the yields of oilseed crops.

Characterization of the genetic diversity in guayule, a natural source of rubber. Guayule is a perennial shrub native to the southwestern United States and Mexico that holds great promise as a sustainable source of natural rubber and hypoallergenic latex. The yields of rubber in guayule, however, are quite low, and thus research is required to increase overall productivity. Modern plant breeding offers a powerful approach to increase rubber yields, but any successful breeding program requires diverse, well-defined accessions that can serve as parental lines. In an effort to characterize the genetic diversity guayule, scientists at the Arid-Land Agricultural Research Center, Maricopa, Arizona in collaboration with Cornell University, the International Maize and Wheat Improvement Center, Kansas State University, and West Virginia University used “genotyping-by-sequencing” technologies to determine the DNA sequences of approximately 70 guayule lines and closely related species. An analysis of the DNA sequences revealed the evolutionary relationships between the individuals and also identified many subtle differences in DNA sequence that could be used as “markers” in plant breeding experiments. The results of these studies provide the most detailed description yet for guayule diversity and can be used in future efforts to increase rubber content of guayule through modern plant breeding.

Assembly of the guayule (*Parthenium argentatum*) genome. The goal of this project is to produce a guayule genome providing the foundation for both genetic and biotechnological improvement of this plant. The complete guayule genome (as determined by read mapping) was computationally assembled employing next generation DNA sequencing technologies. This assembly includes approximately 1Gb of scaffolds containing more than 95% of the guayule genes, and has been verified via syntenic relationships to other dicotyledonous plant genomes and contains a number of unexpected architectural features. The assembly and scaffolds have been uploaded for use cooperators in a USDA-BRDI funded consortium to improve guayule. Following publication within the next year, this genomic sequence will be made publically available for guayule improvement by both public and private sector scientists.

Akron, CO

Brassica napus for use in genome-wide association studies. ARS researchers in Akron, Colorado collected grain yield, yield components and phenotypic data on two replications of 240 accessions of spring planted *Brassica napus* for use in genome-wide association studies (GWAS). Yields ranged between 100 kg ha⁻¹ per ha up to 1800 kg ha⁻¹. In 2014, 11 plots had yields greater than 900 kg ha⁻¹.

(2) Feedstock Production

Ft. Collin, CO

Best management practices for biofuel production evaluated for soil quality. ARS researchers in Fort Collins, Colorado and Lincoln, Nebraska evaluated current (no-tilled corn, *Zea mays* L.) and future (switchgrass, *Panicum virgatum* L.) bioenergy crops and evaluated soil properties in a rain-fed study of nitrogen fertilizer rate and harvest management. When all measured soil parameters were included in the Soil Management Assessment Framework, switchgrass improved soil quality index over time at all depths. No-till corn without residue removal did not improve soil quality index, but 50% residue removal of corn stover decreased soil quality index. Even with best-management practices such as no-till, corn stover removal will have to be carefully managed to prevent soil degradation.

Lincoln, NE

Current and future biofuel feedstock systems grown on marginal lands can reduce greenhouse gas emissions. Low-carbon biofuel sources are being developed and evaluated in the United States and Europe to partially offset petroleum transport fuels. ARS researchers in Lincoln, Nebraska and Ft Collins, Colorado evaluated current and potential biofuel production systems from a long-term study of continuous no-tillage corn (*Zea mays* L.) and switchgrass (*Panicum virgatum* L.) grown on marginal lands. They conducted field trials under differing harvest strategies and nitrogen fertilizer intensities to determine overall environmental sustainability. In a lifecycle analysis, both corn and switchgrass grown for bioenergy reduced lifecycle greenhouse gas emissions as a direct result of soil carbon sequestration deeper than 30 cm and from the adoption of integrated biofuel conversion pathways. A multi-feedstock, landscape approach coupled with an integrated biorefinery would be a viable option to meet growing renewable transportation fuel demands while improving the energy efficiency of first generation biofuels.

DayCent model can accurately predict biomass yields, nitrous oxide emissions and soil carbon stocks of biofuel cropping systems. DayCent simulations of biomass yields and greenhouse gas fluxes were compared with observations from different biofuel cropping systems in Nebraska, Pennsylvania, and Illinois. The model accurately represented biomass yields for switchgrass at all 3 sites and prairie grass and miscanthus

in Illinois, but under-estimated reed canary grass yields in Pennsylvania. In addition, the model showed good agreement with nitrous oxide emissions and soil carbon stock measurements at the Illinois and Pennsylvania sites, and with soil carbon at the Nebraska site. Subsequently, the model was applied to investigate the impacts of winter-spring season production of wheat and barley biofuel feedstocks on soil carbon levels and N₂O emissions. Simulations suggest that including winter/spring season biofuel cropping with residue removal in corn/soy rotations typically increases soil N₂O emissions and nitrate leaching, but soil carbon stocks can be maintained if the high-lignin fermentation byproduct from cellulosic ethanol production is applied to soil.

Maricopa, AZ

Development of direct seeding methods for guayule, a natural source for rubber and biomass. Guayule, *Parthenium argentatum*, is a desert shrub that produces latex in bark tissues and represents a potential domestic source of natural rubber and biomass. One of the major challenges for cultivating guayule, however, is that the plant must be hand-sown using seedlings, which significantly increases the costs and labor associated with crop production. ARS scientists in Maricopa, Arizona, in collaboration with scientists at the University of Arizona, Cornell University, West Virginia University, and Seed Dynamics Incorporated of Salinas, California, developed direct-seeding methods for guayule and optimized conditions for seed germination and seedling establishment. The results of these studies will directly impact the guayule industry and underpin future efforts to further improve guayule cultivation and agronomics.

Parlier, CA

Growing plants with high salinity drainage water. Reductions in water supplies have reduced production in irrigated agriculture in the western U.S. ARS researchers at Parlier, California, have identified plant species, e.g., mustard, poplar-tree clones, and *Opuntia* cactus, adapted to high saline drainage or ground waters. These plants can be used for producing new bio-based products and have economic value for the grower, e.g., biofuel, Selenium (Se) enriched feed and fruit products. These findings improve our ability to develop a sustainable and agronomic-based system on drainage-impacted soils for reuse or use of poor quality waters. The use of alternative salt and boron-tolerant crops that also manage soluble Se from poor quality waters reduces the growers' need to use limited high quality water in the western U.S.

Water use and carbon balance of sugarcane in Hawaii. With average annual precipitation as low as 275 mm on the leeward side of the 36,000 acres of a sugarcane plantation on Maui, water availability is a major limiting factor for sustainable production of biofuel crops in Hawaii. In a two-year field study, ARS researchers from Parlier, California, conducted a series of field, laboratory, and modeling experiments to quantify crop water use and carbon stock balances of sugarcane production on Maui Hawaii. The field data showed that total crop water use for a 2-year sugarcane cropping cycle was 1390 mm. The research also showed that an average of 4.8 kg carbon per meter square area was accumulated by the sugarcane crop during the 2-year growth cycle, and the

distribution of this carbon over time and soil depths varies with sugarcane variety and soil type across the plantation. The findings on water requirement and carbon sequestration provide the needed information for management decisions on resource sustainability to produce biofuel feedstock in Hawaii.

Crop production in high saline and boron laden soils. A 10 ha field of mustard was established for biofuel production on excessively high saline and boron laden soils. In all studies, uptake and speciation of selenium (Se), boron (B), sodium (Na) and chloride (Cl) was analyzed and measurements were made to determine the amount of Se removed from the Se-laden soil to a depth of 1 meter. Approximately 15% of Se net losses in the saline soil were accounted for in plant material and approximately 20% was assumed lost by leaching. Due to the severity of soil salinity (ranging from 6 to 37 dS/m), and soluble B (ranging from 6 to 27 mg B/L), and soluble Se (ranging from 0.05 to 0.10 mg Se/L and most importantly a lack of any precipitation, Brassica seed yields were dramatically decreased. Yields ranged from a low of 0.8 tons/ha to a high of only 2.2 tons/ha. Analyses showed that excessive accumulation of B and Na in the soil and subsequently in the plant tissue and a lack of precipitation significantly contributed to decrease in seed yields. Seed processed for oils did not produce more than 500 liters of 100% biodiesel per ha.

Akron, CO

Feedstock production in dryland rotational systems. ARS researchers in Akron, Colorado are evaluating two four year, no-till rotations: Wheat-Corn-Millet-Canola (WCMCa) and Wheat-Canola-Corn-Fallow (WCaCF). The primary focus of was on yields, soil water use, and N use. Total N of the grain samples and the oil content of the canola was also measured. The crops harvested in 2014 (2013-2014 crop year) had more favorable precipitation resulting in better crop yields. Comparing the yields for the two rotations, the WCaCF rotation had better yields for all crops than the WCMCa rotation in 2014. This we would expect since corn follows wheat in the WCMCa rotation allowing slightly more time for soil water storage and also more residue lowering evaporation and increasing snow catch compared to following canola in the WCaCF rotation. In 2014, all crop yields were average to above average with the exception of the canola in the continuously cropped WCMCa rotation. In 2015, the canola yields were exceptional for both rotations. The wheat was 13 bushels better in the plots with fallow (WCaCF). A hail storm in early August 2015 damaged the corn and millet reducing the yield potential of both crops. The breakeven yields for canola at current prices is 800 lbs/acre. The last two years that yield has been achieved 75% of the time. Breakeven yields for corn is 35 bushel/acre. Breakeven yields for wheat is 30 bushel per acre. The last two years the yields have all been favorable and mostly at levels that are economically sustainable. The WCaCF rotation has the best wheat and canola yields in 2014, in 2015 the canola yields were about the same for both rotations. It is an open question if the rotation with fallow is better economically than WCMca. The millet crop in the WCMca is perhaps good enough to compensate for the lower wheat, and sometimes canola yields seen with this rotation.

(3) Conversion and Co-product Utilization.

Ft. Collins, CO

Biochar potentially stimulates soil microbial activity and growth. Biochar amendment to soils is a proposed strategy to improve soil fertility and mitigate climate change, but before this can become a recommended management practice a better understanding of the impacts of biochar on the soil biota is needed. ARS researchers in Ft Collins, Colorado in collaboration with Colorado State University determined the effect of addition rates of a fast-pyrolysis wood-derived biochar on the extraction efficiency, abundance and temporal dynamics of microbial community biomarkers in four temperate soils during a 1-year incubation. Biochar addition proportionally increased microbial abundance in all soils and altered the community composition, particularly at the greatest addition rate, towards a more gram-negative bacteria dominated (relative to fungi and gram-positive) community. Overall, our study provides support for biochar use as a soil amendment that potentially stimulates microbial activity and growth.

Expansion of the Greenhouse gas Reduction through Agricultural Carbon Enhancement network (GRACenet) and Renewable Energy Assessment Project (REAP) databases. The publically accessible portion of the GRACenet and REAP database, which was made available in early 2013, continues to expand and currently houses more than 90,000 greenhouse gas measurements from various cropping and grazing systems across the US going back to the 1980's. In addition, the system includes detailed records of ancillary measurements (e.g., crop yields, soil carbon, nitrogen, water content) as well as information needed to develop and test agro-ecosystem models (e.g., daily weather, land management, soil physical properties). To date, 61 registered users have performed 83 data downloads. These data have been used to validate the DayCent model, which is used to calculate soil greenhouse gas emissions for the US Greenhouse Gas Inventory compiled annually by the EPA. The system has also been made available to those interested in sustainability of bioenergy feedstocks through a linkage to the DOE's Knowledge Discovery Framework (KDF). The GRACenet and REAP data sets are also linked with the USDA data portal (<http://nrrc.ars.usda.gov/arsdataportal/#/Home>), which is intended to accommodate different USDA projects and is GIS referenced.

Albany, CA

Torrefied crop waste as a replacement for carbon black. Carbon black is used as a filler in plastic composites to improve the heat resistance and appearance. ARS scientists at the Western Regional Research Center in Albany, California used ground torrefied crop waste from almond shells and wood chips as a replacement filler for carbon black. The composites had greater heat stability than the plastic by itself but the strength and elongation of the composites, although adequate for many applications, were inferior. The results indicate that torrefied crop waste could be a viable filler for plastic composites for many applications.

Low cost treatments for making fibrillated nanofibers. Fibrillated nanofibers have been shown to improve the strength of plastic composites but are prohibitively expensive. ARS scientists at the Western Regional Research Center in Albany, California in collaboration with Brazilian scientists have demonstrated that degradative enzymes from crude digestate are effective in making fibrillated nanofibers from pulp fiber. Different incubation times yielded correspondingly varied degrees of fibrillation. This research could lead to greater production of low-cost fibrillated nanofibers that can improve the strength of fiber composites and increase their renewable material content.